JPL Electronic Nose: From Sniffing Brain Cancer to Trouble in Space

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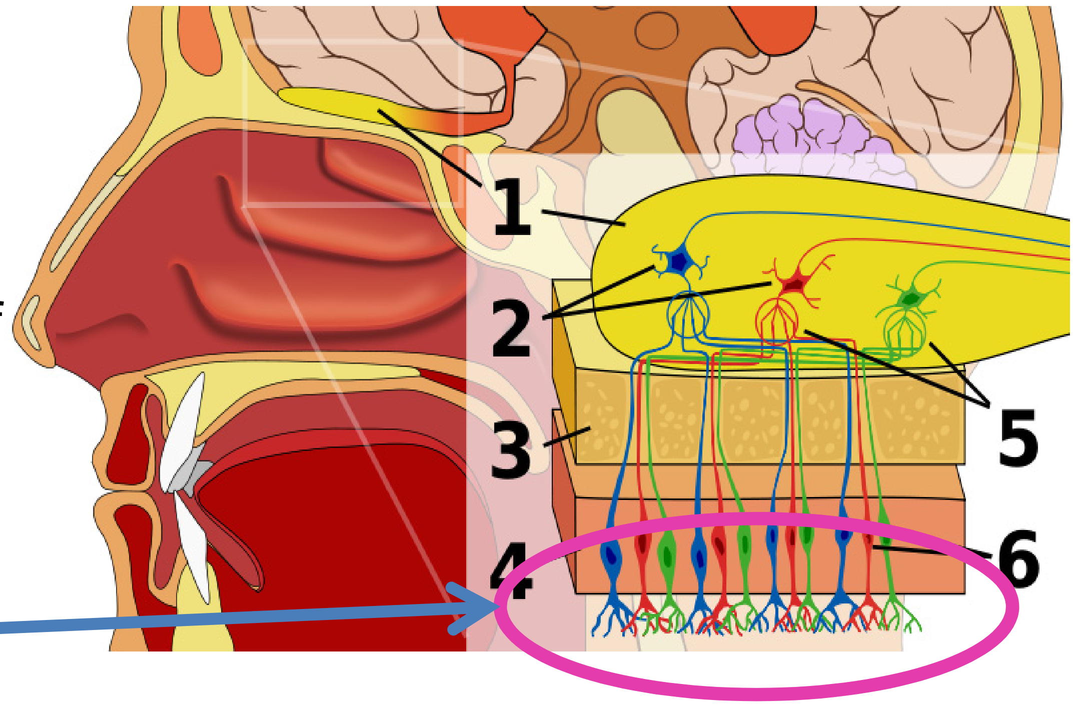
Human Olfaction:
Our inspiration

The Nobel Prize in Physiology or Medicine 2004 went to Richard Axel, Linda B. Buck

for their discoveries of "odorant receptors and the organization of the olfactory system"

Olfactory receptor cells are non-specific sensors.



Human olfactory system. 1: Olfactory bulb 2: Mitral cells 3: Bone 4: Nasal epithelium 5: Glomerulus (olfaction) 6: Olfactory receptor cells

- no acetone receptor cell
- no orange juice receptor cell

Chemical Sensing:
"Lock and Key" Model

Receptor-Molecule interaction

shape specific

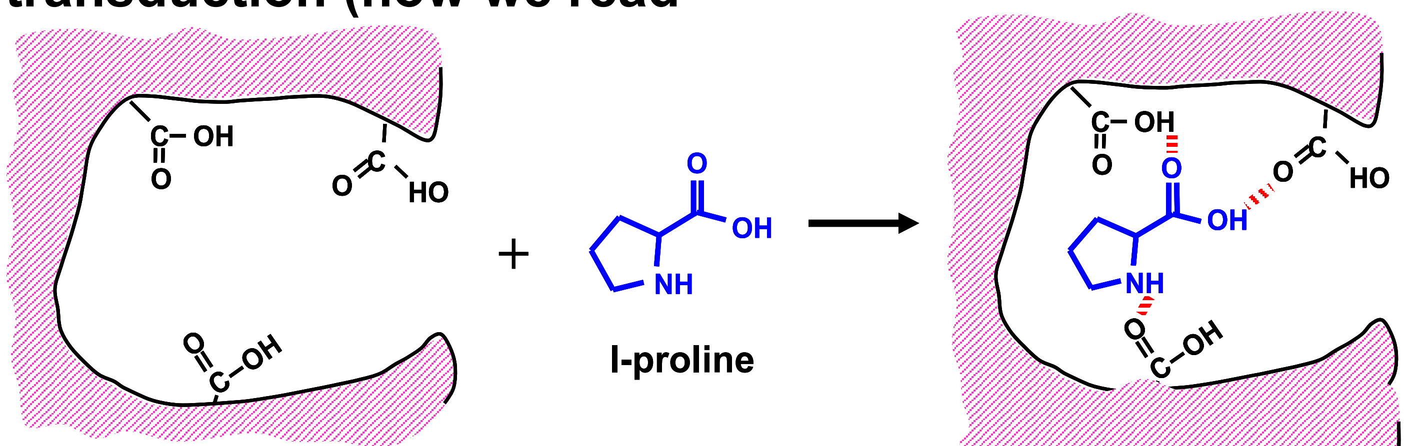
charge specifc

Response and transduction (how we read the response)

electrical

optical

mechanical

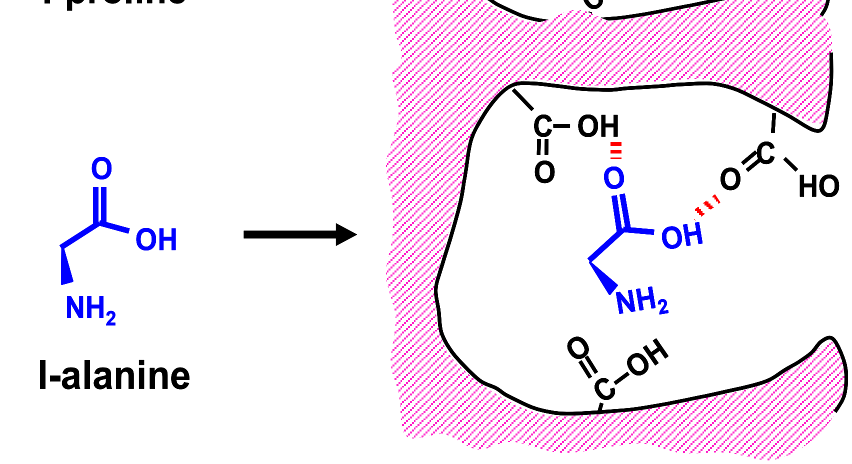


Very few receptors are truly selective

cross-sensitivities

false positives, negatives

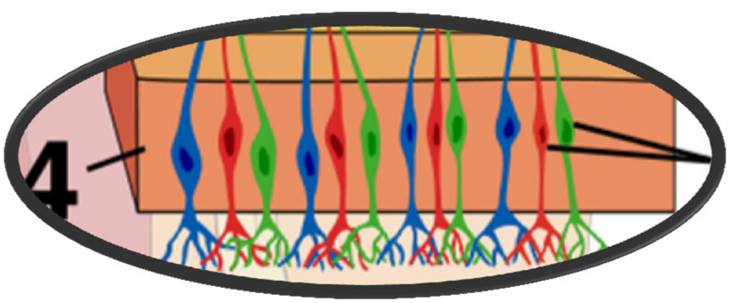
errors in quantitation



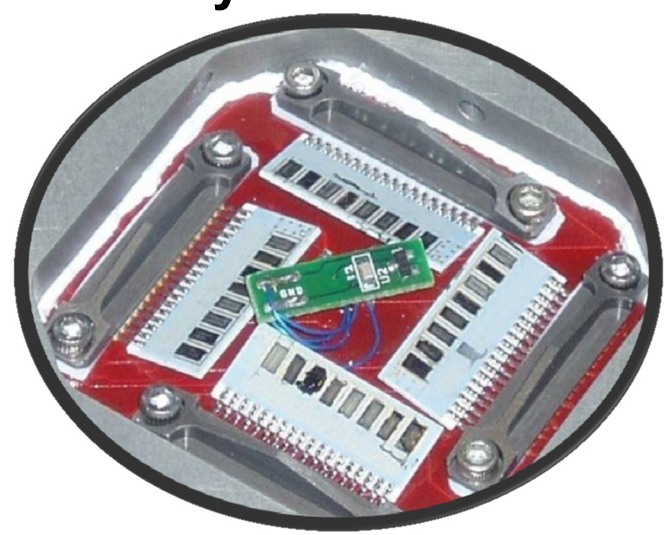
Chemical Sensing:
Sensor Arrays

Array-based sensing utilizes cross-sensitivities

- **Elements in array are semi-selective toward target molecules**
- **Array responds in "fingerprint" pattern to stimulus**
- **Response of entire array is used to identify stimulant**



Olfactory receptors



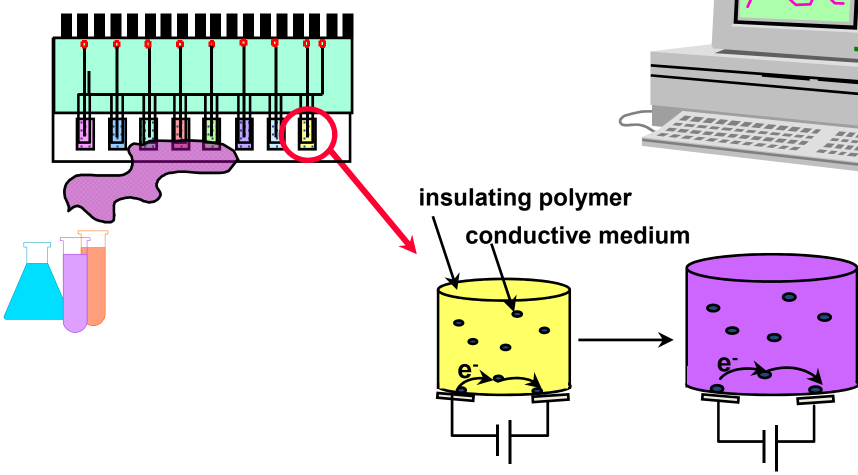
JPL Sensing Array
polymer-carbon composite sensors

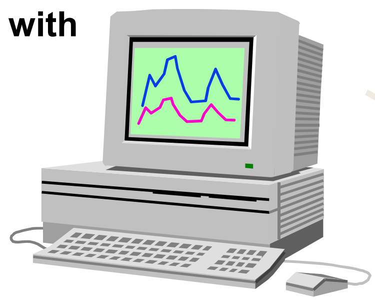
What Is An Electronic Nose?

An array of non-specific chemical sensors, controlled and analyzed electronically, which mimics the action of the mammalian nose by recognizing patterns of response

1. **ENose measures background resistance in each sensor and establishes a baseline.**

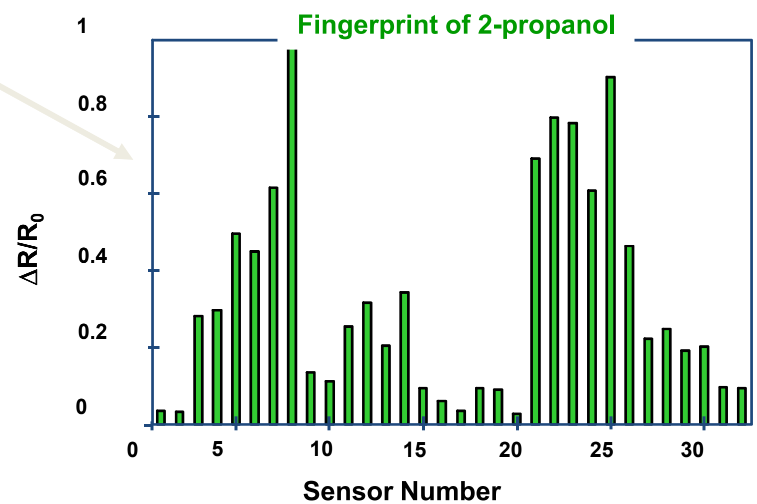
2. **Contaminant comes in contact with sensors on the sensing head.**





3. **The sensing films, change physical properties, such as thickness or color, as air composition changes.**

4. **Sensor response is recorded by a computer, the change in resistance is computed, and the distributed response pattern of the sensor array is used to identify gases and mixtures of gases**



5. **Responses of the sensor array are analyzed and quantified using software developed for the task.**

propanol 300 ppm

Air Quality Monitoring For NASA

Long-Duration Space Flight Requirements

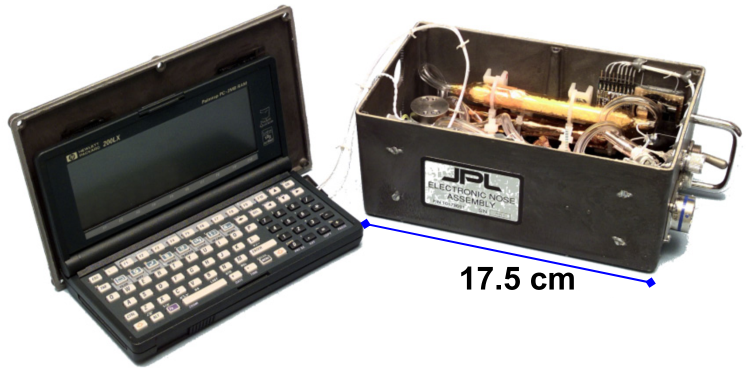
- High level of crew productivity; little habitat maintenance
- Decouple environmental control from ground control
  - Distributed network of sensors and actuators
  - Sound an alarm and/or actuate remedial action
  - Early identification of areas requiring remediation

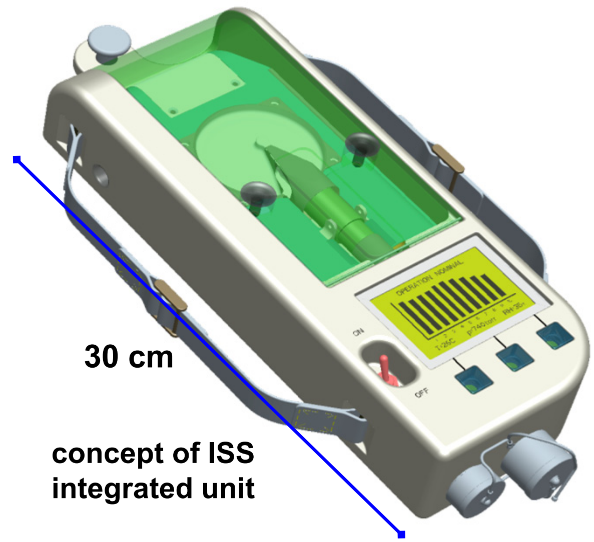
Electronic Nose Operational Purpose

- Incident monitor for contaminants exceeding Spacecraft Max Allowable Concentration (SMAC)
  - Alcohols (contaminates air handling system)
  - Mercury (in light bulbs)
  - Freon, ammonia (in cooling loops)
  - Sulfur dioxide (in batteries)
- Monitor clean-up process

JPL Electronic Nose (ENose)

Three Generations of Autonomous ENoses





concept of ISS integrated unit

Generation 1

Experiment on STS-95, TRL 6-7

Volume: 2000 cm^3 inc. computer

Mass: 1.4 kg including computer

Power: 1.5 W ave., 3 W peak

Detect/ID/Quant 10 compounds at 1 hour SMAC. No real-time data analysis; data acquisition and device control with HP 200LX computer.

6 day flight experiment successful.

Generation 2

Ground Testing, TRL 5-6

Volume: 750 cm^3 w/o computer

Mass: 800 g w/o computer

Power: 1.5 W ave., 3 W peak

Detect/ID/Quant 21 compounds at 24 hour SMAC. Data acquisition and device control possible with PDA computer; real time data analysis with ultra micro computer.

Extensive ground testing in environmental chamber.

Generation 3

SDTO Exp. on ISS, TRL 4

Volume: ~3000 cm^3 inc. computer

Mass: 1.5-2 kg inc. computer

Power: ~ 4 W ave., ~ 10 W peak

Detect/ID/Quant 10 compounds at defined concentrations, including Hg, SO_2. Deconvolute mixtures, id unknowns by functional group.

Data acquisition, device control, real time data analysis included.

Extensive ground testing in environmental chamber followed by six month test on-orbit.

Medical Applications of Electronic Noses: Advantages and Limitations

Advantages of Diagnosis with an Electronic Nose

- Instruments are portable, in some cases hand-held, relatively inexpensive and do not require extensive training or laboratories for use
- Analysis of data can be accomplished in several minutes
- Sample collection is simple
  - vapor phase samples drawn directly into device *or*
  - fluids placed into receptacle and headspace sampled *or*
  - take swab, hold in vitro 30-120 minutes, moderate or no heat
- NASA is interested for long-duration space flight

Limitations of Diagnosis with an Electronic Nose

- Studies generally have samples of 10-100 patients and so do not yet show general applicability
- Breath analysis requires careful adjustment to water vapor
- Several studies tried multiple approaches to data analysis before finding one which gave high correlation with control or other clinical data
- Marker compounds for diseases are not always well characterized; use for diagnosis of different diseases will require different sets of sensors and specific data analysis routines
- Other odors may interfere with sensor response

Clinical Studies Using an Electronic Nose: Sample types

Breath Analysis

Exhaled breath is collected in a sampling bag or patient breathes directly into device. Exhaled breath may contain several tens of volatile organic compounds as well as water vapor

Swab Headspace Analysis

Liquid (sweat, sputum, infection site) collected on a swab; swab enclosed and headspace sampled to detect bacterial metabolic products.

Odor Analysis

Device used to "sniff" patient to detect chemical species which signal diseases

Culture headspace analysis

Headspace above a culture plate is sampled to detect metabolic or other products

Medical Applications of Olfaction: *Diagnoses using breath or fluid analysis*

Studies Using Breath Analysis for Diagnosis

Pneumonia (VAP) ***80% accuracy***

- **detection of VOC markers**
- **correlated with CT scans, other clinical data**

Diabetes mellitus

- **detection of acetone**
- **correlated with clinical data**

Studies Involving Fluid Analysis (sweat or swabs)

Schizophrenia **>95% accuracy**

- **variation in body odor**
- **discrimination from other mental illness and controls**

Tuberculosis **90% accuracy**

- **bacterial metabolic products in vitro or swab headspace discrimination among various bacteria**
- **correlated with cultured swabs**

ENT Infection (S. aureus) **88% accuracy**

- **swab headspace discrimination among three bacteria subclasses**
- **correlated with cultured swabs**

Electronic Nose:
Sniffing Brain Tumors

Olfactory Detection of Cancers

Why Would Tumors Have An Odor Signature?

- **Studies of volatile organic compounds produced by tumors show it is possible to classify by VOCs**
- **Studies of differences in protein structure of cancerous and normal tissues**
- **Studies of volatile organic markers in breath of cancer patients**
- **Studies for detection of lung cancer using two different e-noses; 70 - 90% accuracy**
  - **detection of VOC markers**
  - **correlated with clinical data**

Tumor-Sniffing Dogs

- **Studies of canine detection: lung, breast, bladder, skin**
- **Trained dogs detect presence of cancer through breath, fluid or direct odor analysis. High level of accuracy (85-95%)**

MEDICAL APPLICATIONS OF ELECTRONIC NOSES

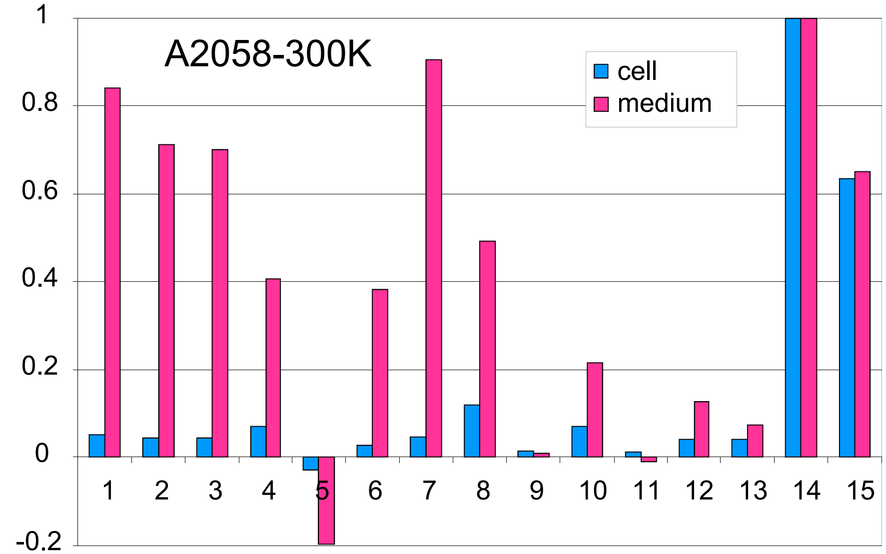
In vitro Studies

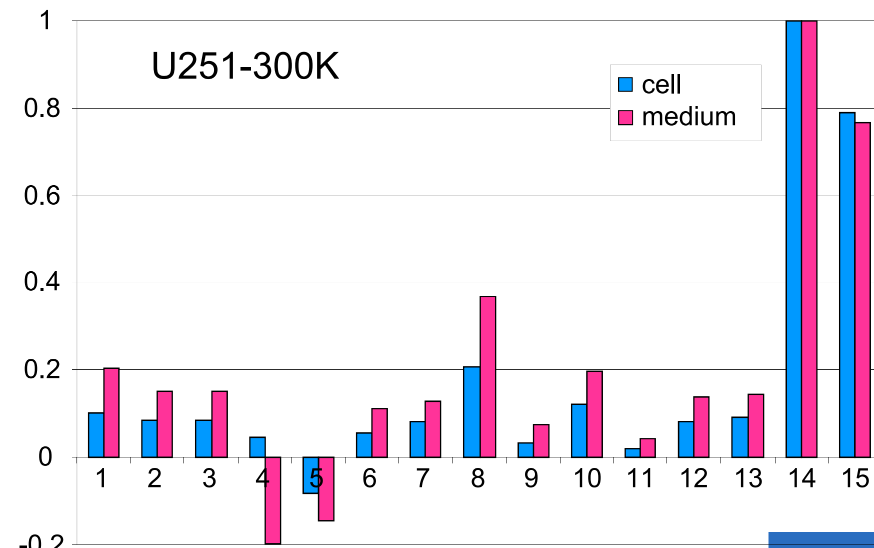
Step 1: Can cultured cells be distinguished from the medium in which they are cultured?

By looking at the "fingerprint" pattern of the sensing array, we can see differences in the pattern. These differences indicate that cultured cells can be distinguished from the medium in which they are cultured.

The upper plot is cell culture A2058 (melanoma cell line) compared with its growth medium; the lower plot is cell culture U251 (brain tumor cell line) compared with its medium. There are significant pattern differences for cells with medium decanted (blue) and medium alone (red).

Magnitudes have been normalized to the largest sensor signal in the array to show response pattern without concentration dependence. Error on each sensor response is ~ 5%.





MEDICAL APPLICATIONS OF ELECTRONIC NOSES

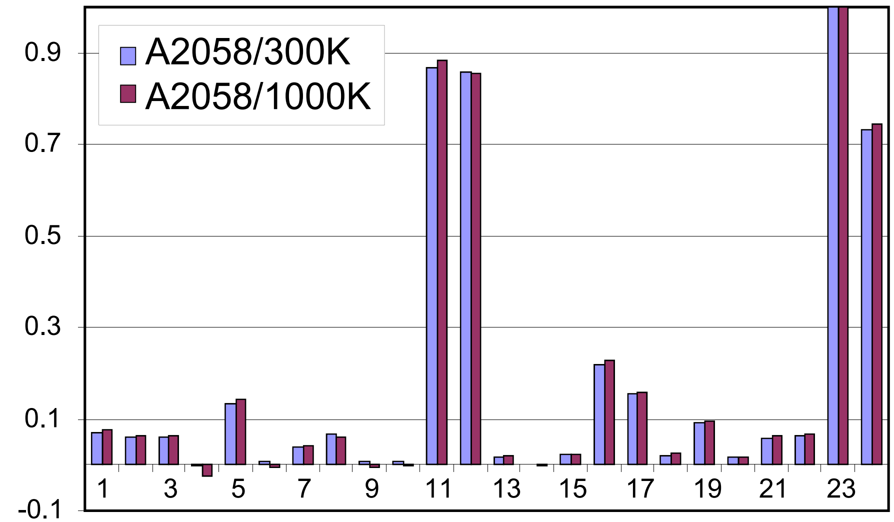
In vitro Studies - Tumors

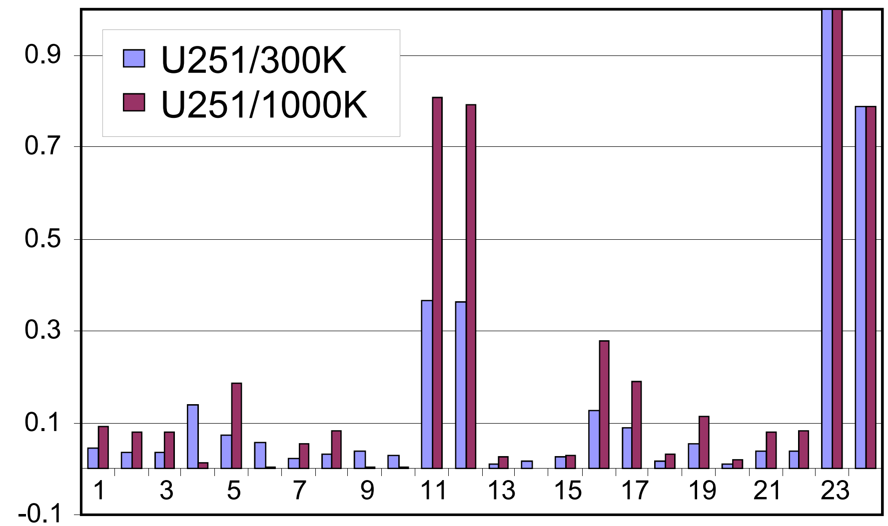
<u>Step 2:</u> Does the number of cells in a culture influence the odor fingerprint?

The fingerprint pattern of the sensing array in the upper plot, shows that for A2058 (melanoma) cells, there is no significant difference in array pattern for 300,000 and 1,000,000 cells. The total variation across a 24 element array is 8%. Variation less than 10% is not sufficient to distinguish array signal.

The lower plot shows a much greater difference by cell number for U251 (brain tumor) cells. The difference in array pattern is 49% for 300,000 vs. 1,000,00 cells.

Sensor response magnitudes have been normalized to the largest sensor signal in the array to show array fingerprint.





MEDICAL APPLICATIONS OF ELECTRONIC NOSES

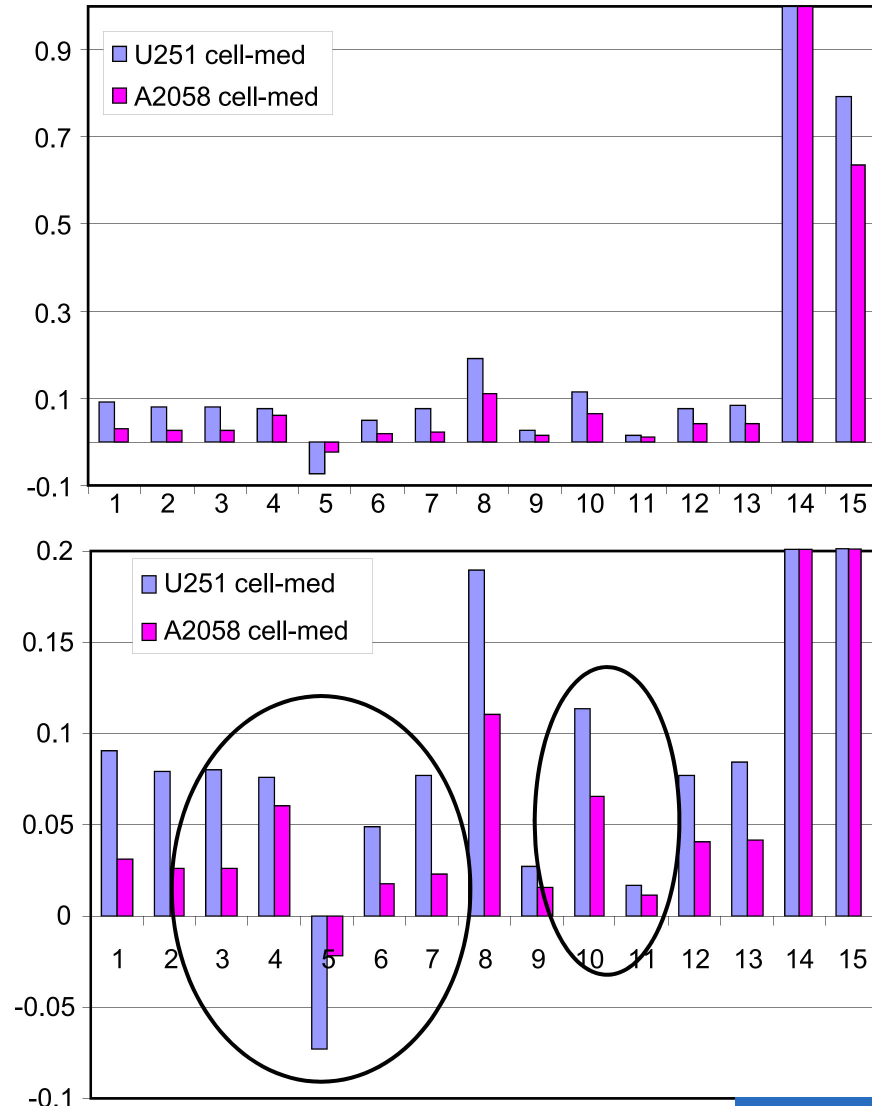
In vitro Studies - Tumors

Step 3: Can two different types of tumor cells be distinguished?

This plot compares the "fingerprints" of two types of cells which were cultured in different media. A2058 are a melanoma cell line; U251 are a brain tumor cell line. Signal caused by the growth medium has been removed.

The lower plot is an expansion of the overall, upper plot. Fingerprint pattern differences are judged by the relative magnitudes of sets of sensors. Pattern differences which will allow distinction are shown in circled regions.

The variation across the 15 element array 18%.



MEDICAL APPLICATIONS OF ELECTRONIC NOSES

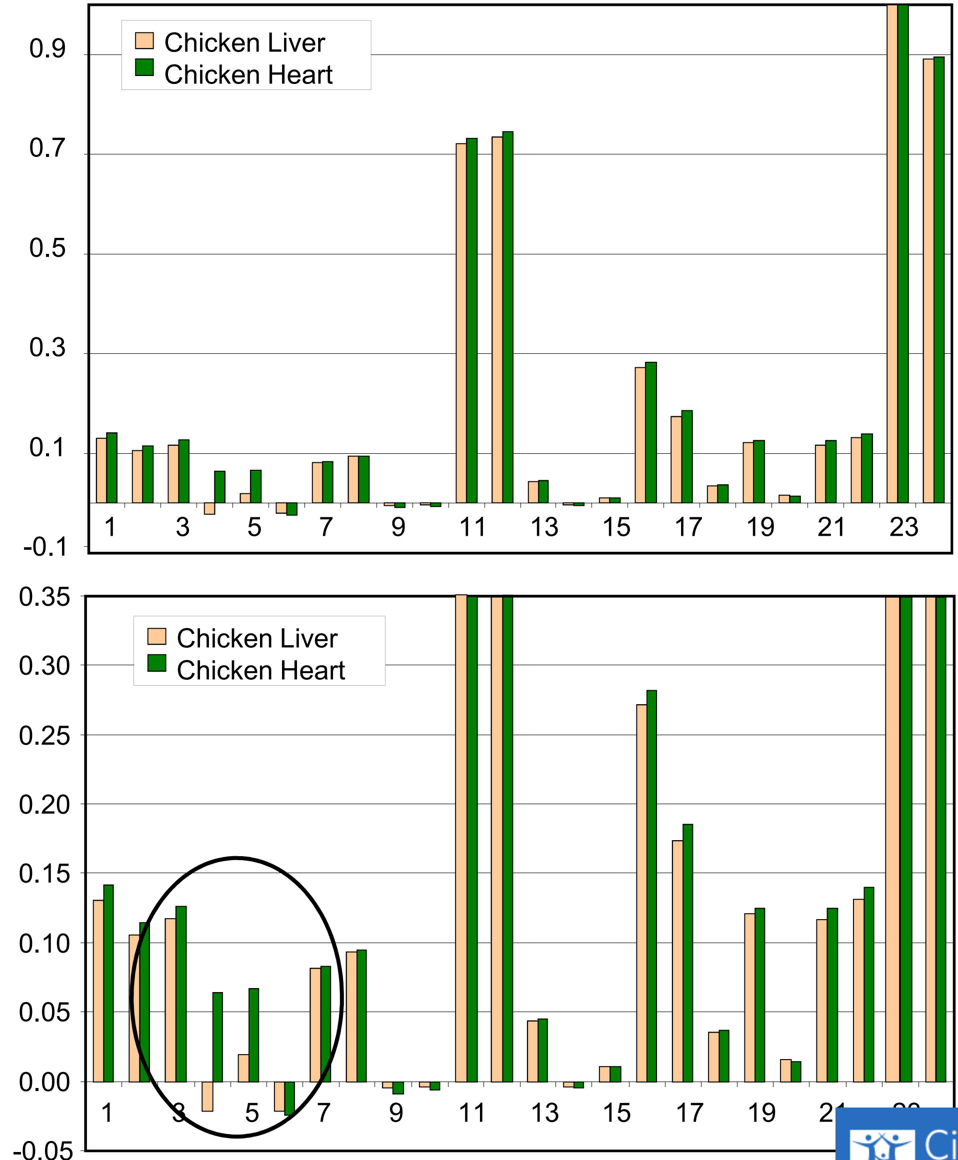
In vitro Studies - Tissue

Step 4: Can two different types of tissue be distinguished?

This plot compares the fingerprints of two types of tissue - chicken liver and chicken muscle (heart).

The lower plot is an expansion of the overall, upper plot. Differences which will allow distinction are shown in circled regions.

The variation across the 24 element array is 19%.



MEDICAL APPLICATIONS OF ELECTRONIC NOSES

Preliminary Study for Tumor Detection Using JPL ENose

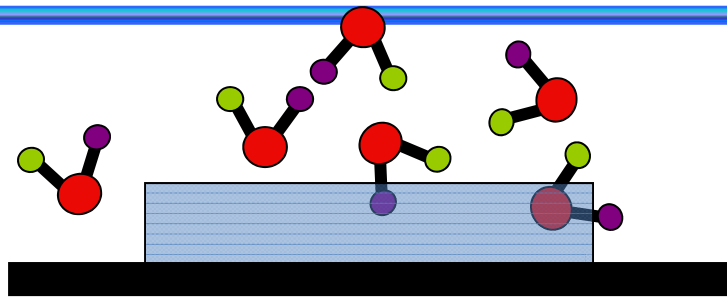
Objective: Determine whether normal and abnormal tissues can be distinguished using an electronic nose

In vitro studies of cultured cells are promising.

JPL ENose was able to distinguish

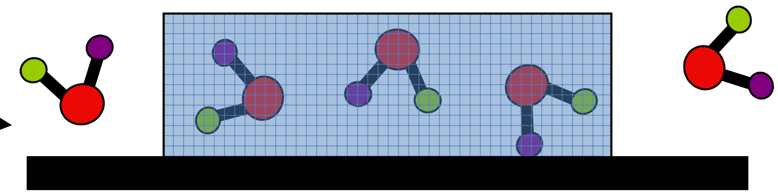
- cells and cell culture medium
- brain tumor cells from "normal" cells
- two types of brain tumor cells
- liver tissue and muscle

How does sensing work?

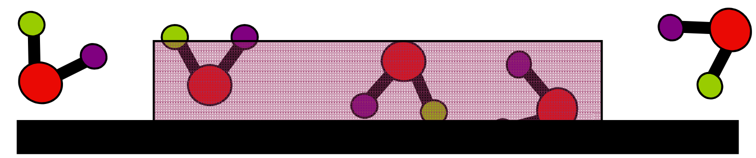


Sensor is exposed
to an analyte

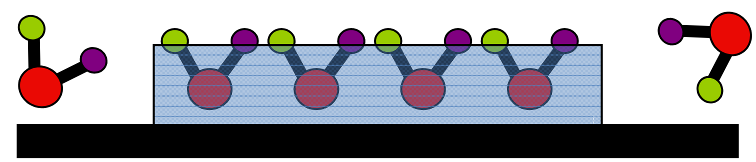
Possible sensor responses



Sensor thickness change

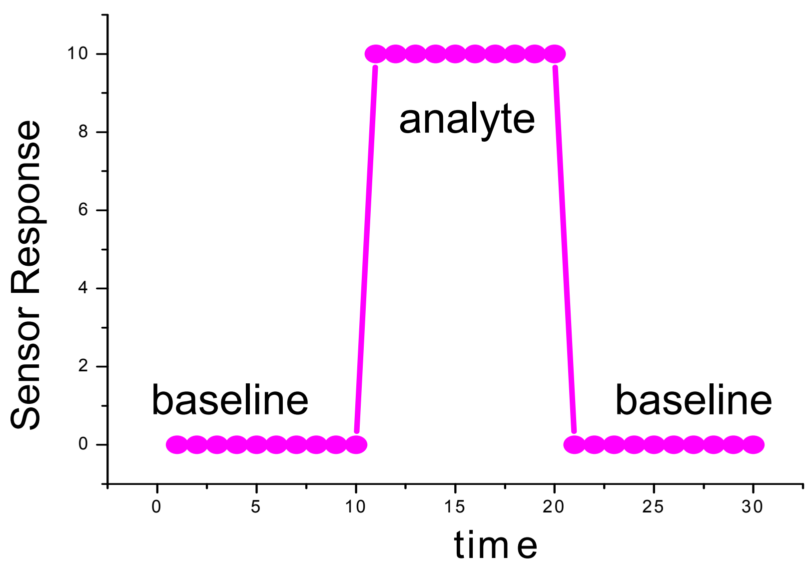


Sensor color changes

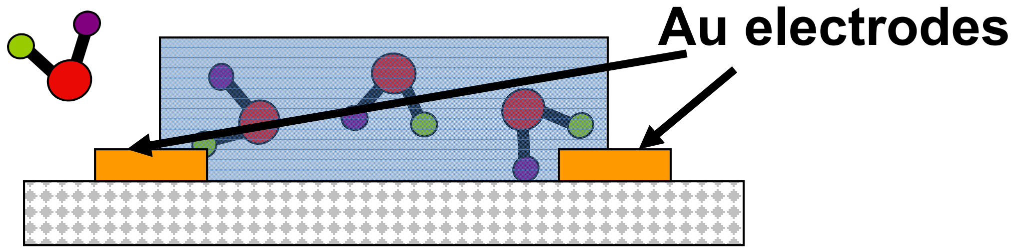


Sensor polarization changes

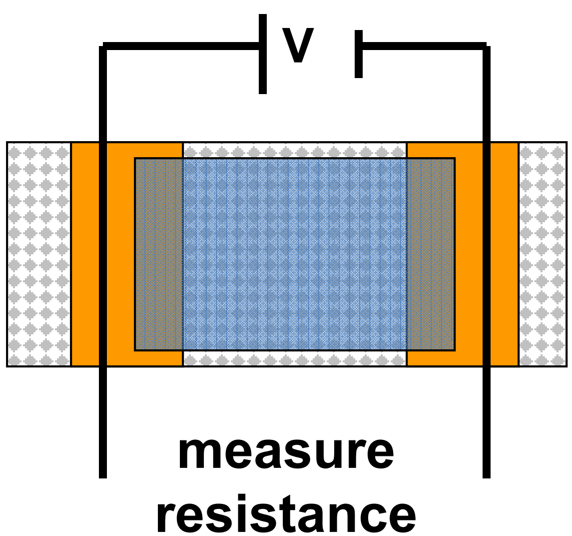
Ideal Sensor Data



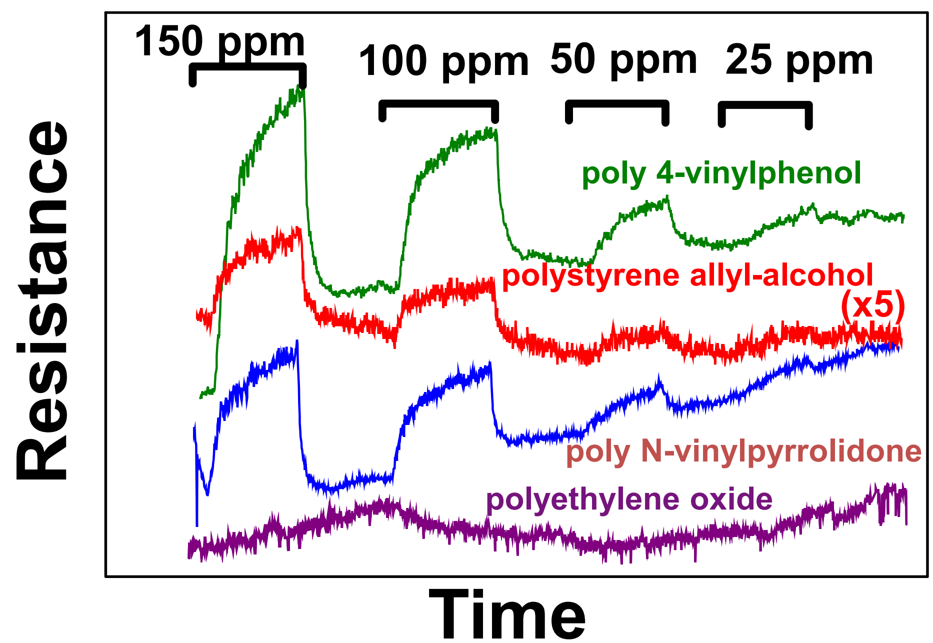
Measuring Sensor Signal: Resistance Readout



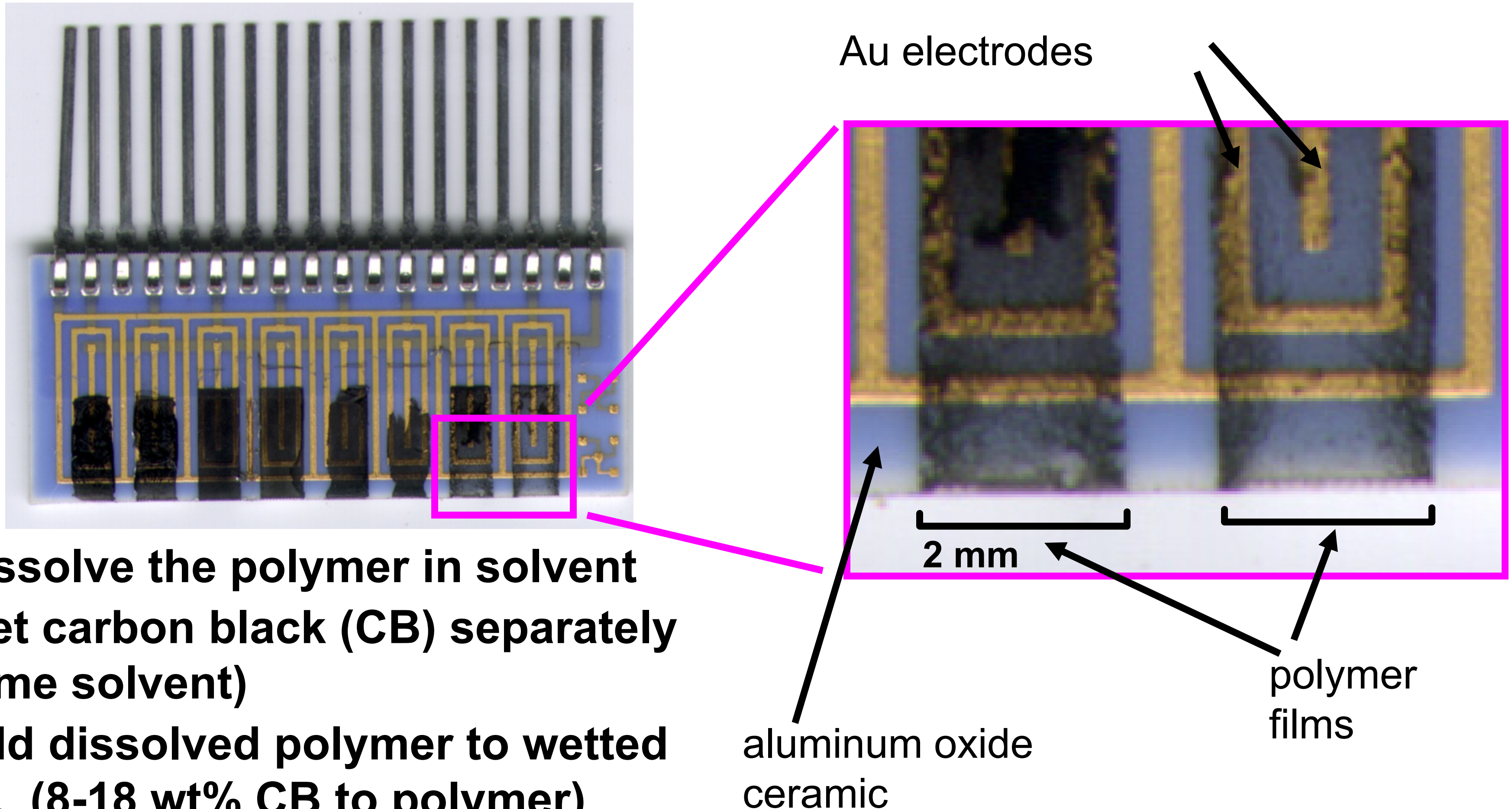
Sensing mechanism: Polymer captures target species and changes the film's conductivity. This leads to a measured change in resistance.



Polymer Film Responds to Methanol

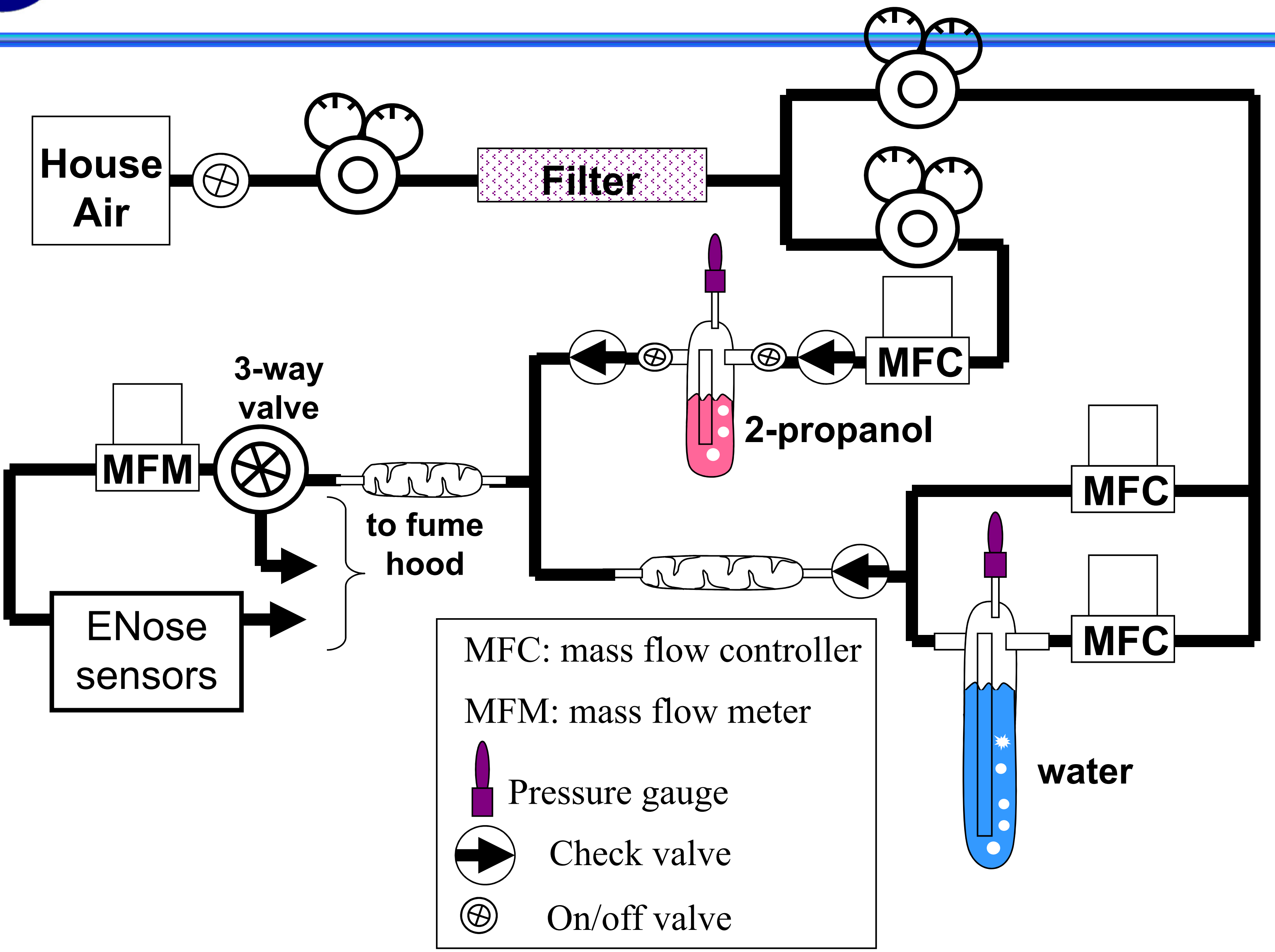


Sensor Preparation: Polymer-Carbon Black Composites

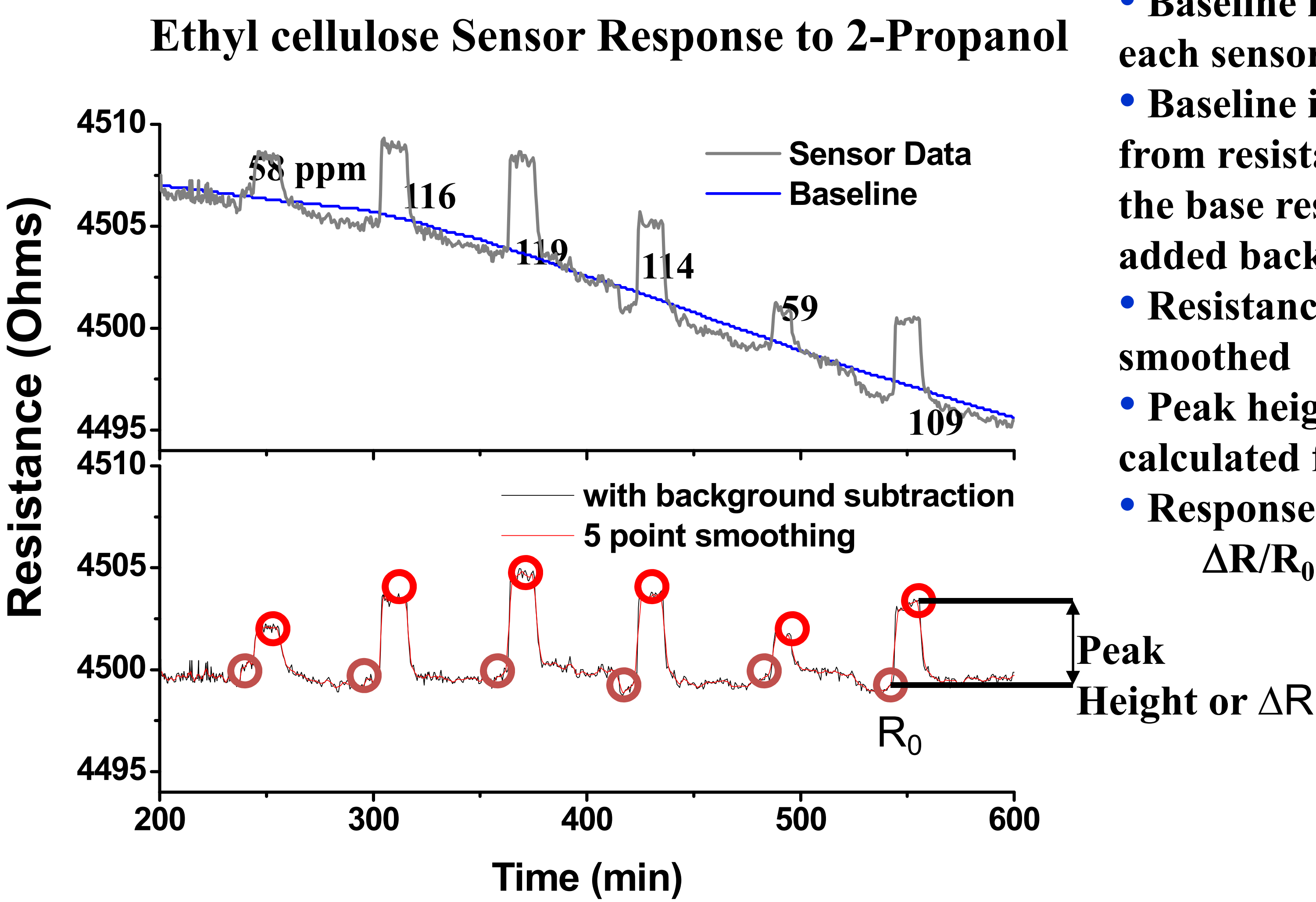


- Dissolve the polymer in solvent
- Wet carbon black (CB) separately (same solvent)
- Add dissolved polymer to wetted CB. (8-18 wt% CB to polymer)
- Sonicate suspension
- Deposit/pipette on to ceramic substrate

Experimental Set-up for Sensor Testing

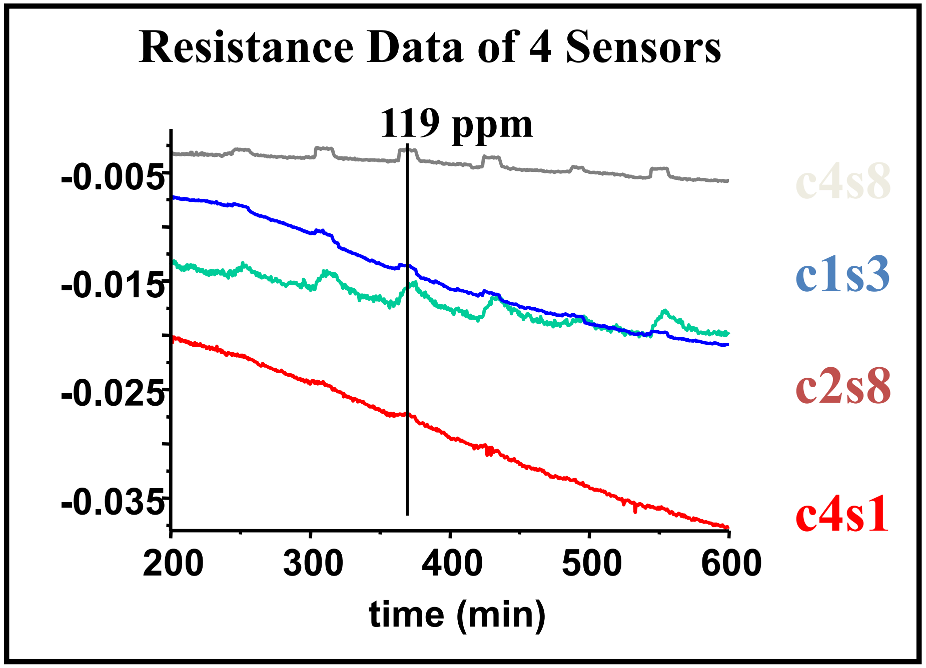


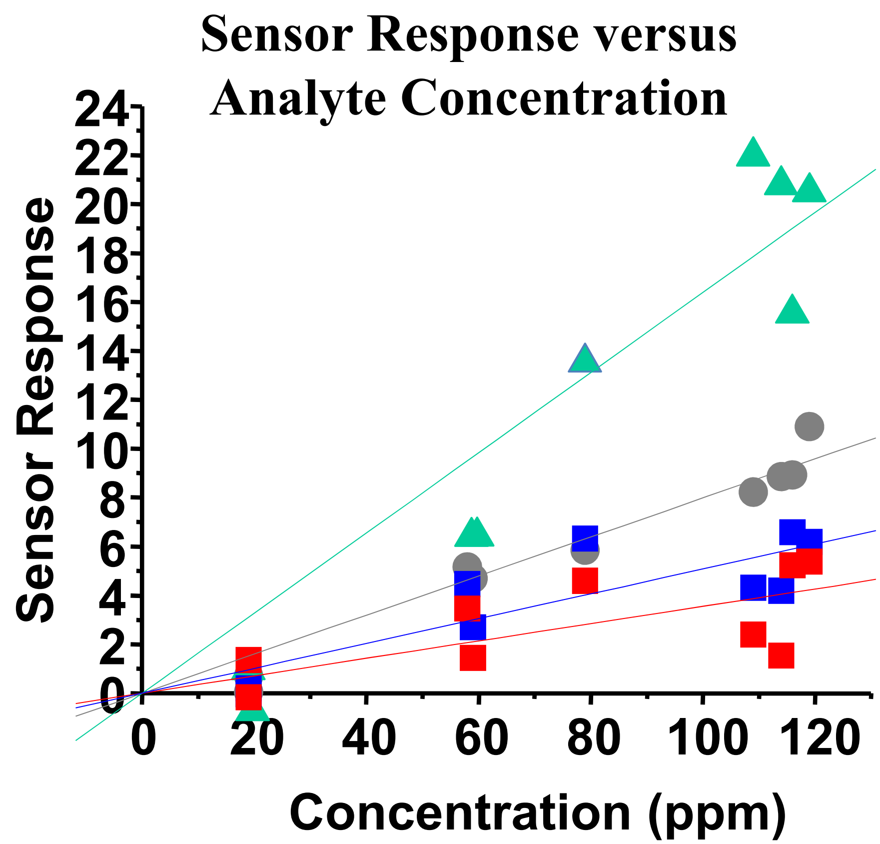
Preprocessing Sensor Resistance Data



- Baseline is generated for each sensor
- Baseline is subtracted from resistance data, and the base resistance is added back in
- Resistance data is smoothed
- Peak height is calculated for each event
- Response defined as $\Delta R/R_0$

Polymer Responses to 2-propanol: Sensor Response Curves

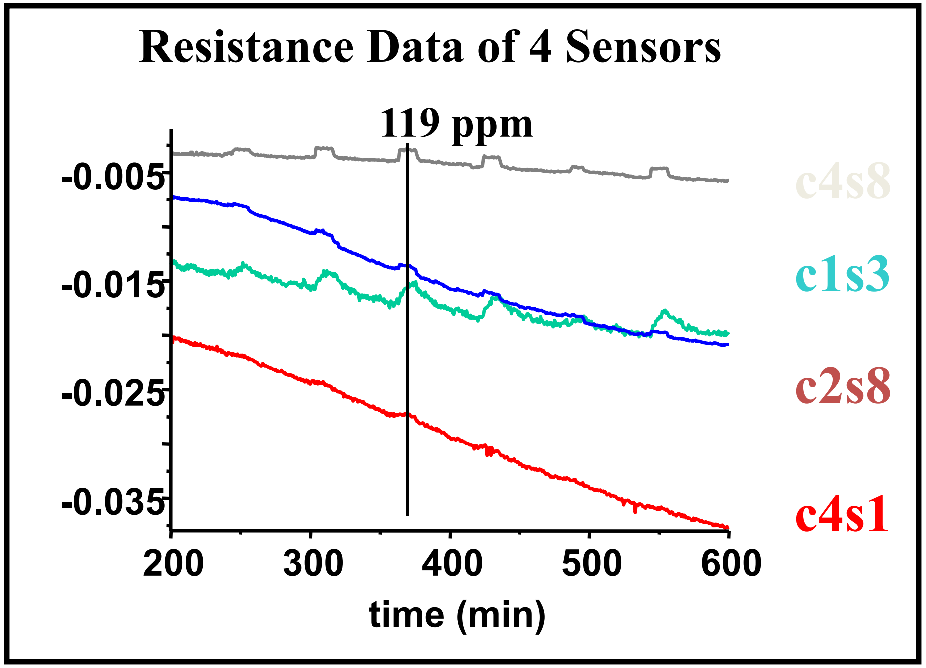


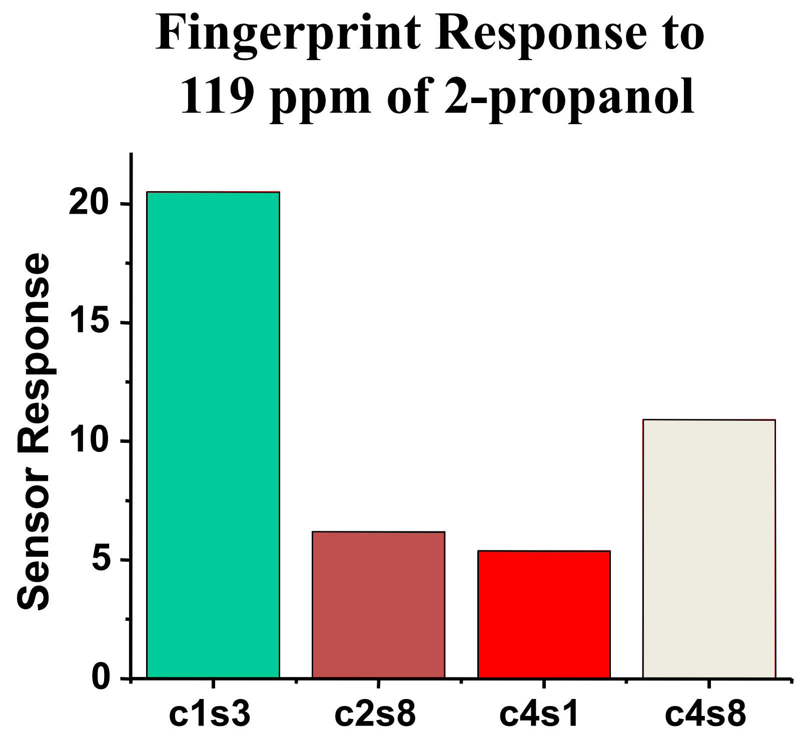


Peak Heights generated from the resistance data can be used to generate sensor response curves.

c1s3 = poly 4-vinyl phenol
c2s8 = polyethylene oxide
c4s1 = Methyl vinyl ether/ maleic acid copolymer
c4s8 = ethyl cellulose

Polymer Responses to 2-propanol: Array Fingerprint





Peak heights generated from the resistance data can be used to generate array fingerprints.

c1s3 = poly 4-vinyl phenol
c2s8 = polyethylene oxide
c4s1 = Methyl vinyl ether/ maleic acid copolymer
c4s8 = ethyl cellulose

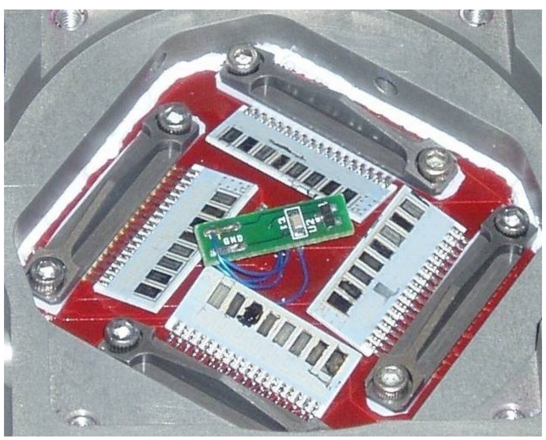
Identification and Quantification of Analytes

Sensor Calibration and Characterization

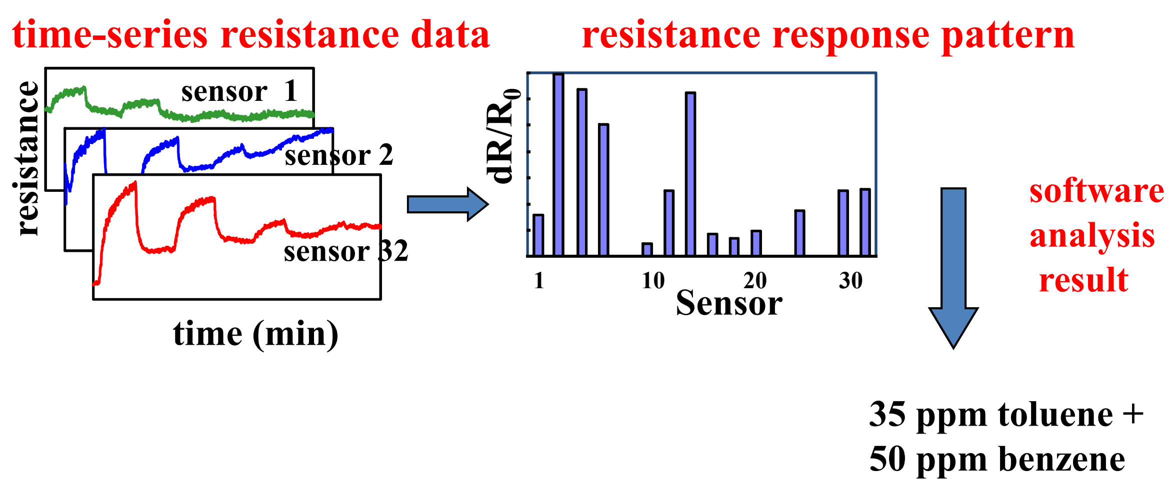
- **<u>Training Sets:</u>** Generate time-series resistances for sensors with known gases and concentrations
- **<u>Preprocess resistance data:</u>** Convert time-series resistance data to resistance response pattern
- **<u>Establish</u>** "fingerprints" for analytes

Identification and Quantification of Analytes

- **<u>Testing Sets:</u>** Generate time-series resistances for sensors with unknown gases and concentrations
- **<u>Preprocess resistance data:</u>** Convert time-series resistance data to resistance response pattern
- **Deconvolute response pattern** by pattern recognition using Levenberg-Marquardt Nonlinear Least Squares Fits (LMNLS)



JPL ENose sensor array (32 sensors)



ENOSE TECHNOLOGY DEMONSTRATION

ISS Data Analysis Summary

- Humidity varied 1- 4% over periods of ~140 minutes; most variation was regular (144 minutes) and was tentatively attributed to the Carbon Dioxide Removal Assembly
- Frequent humidity variation of 3-4% between 12:00 and 17:00; attributed to crew activities, but correlation of times with activities was not possible
- Little ambient temperature variation
- Little ambient pressure variation
- Several events from the ENose target list, 20 – 120 minutes long, detected in 7 month period

JPL Electronic Nose (ENOSE)

ENose Generation 3

Technology Demonstration on ISS funded by ESMD/AEMC

Volume	3.6 L
Mass:	3.4 kg
Power	12 W avg, 20 W peak

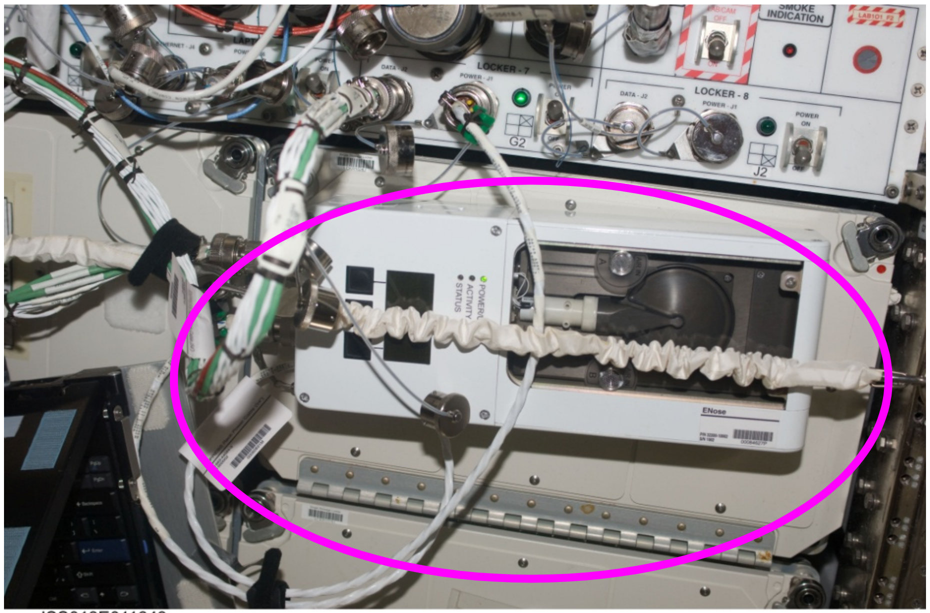
Detect/ID/quantify

Analytes at defined concentrations in environmental conditions of ISS

- acetone
- ammonia
- dichloromethane
- ethanol
- formaldehyde
- Freon 218
- mercury
- methanol
- 2-propanol
- sulfur dioxide
- toluene

6 month test on-orbit in ISS
launch on STS-126; Nov. 14, 2008
activated Dec. 9, 2008

JPL Electronic Nose (ENOSE) Deployed on EXPRESS Rack 2 / ISS



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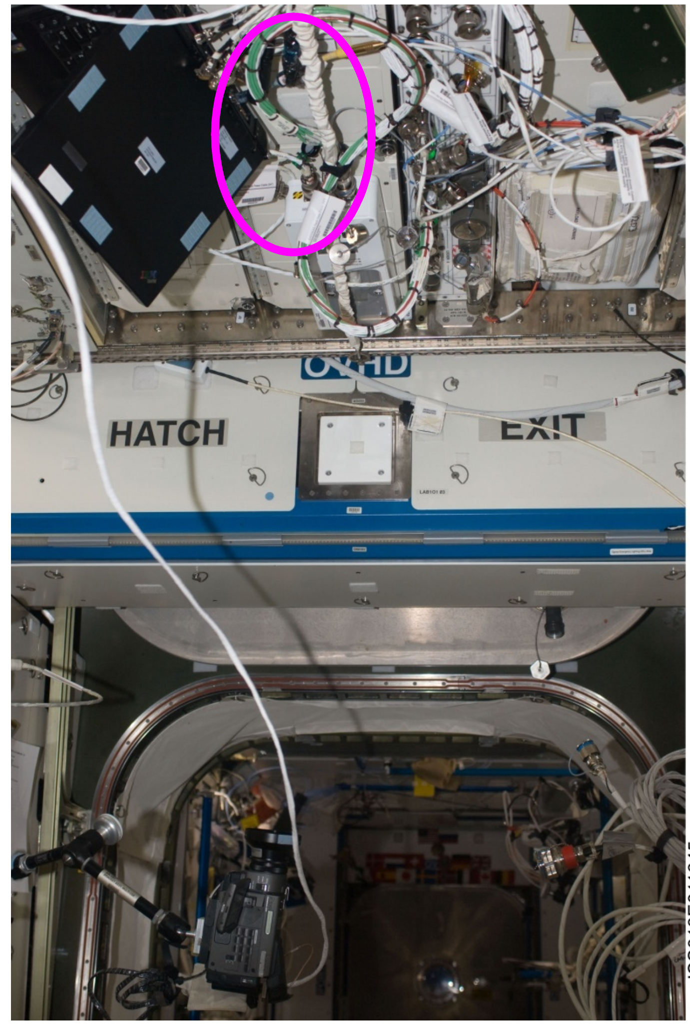

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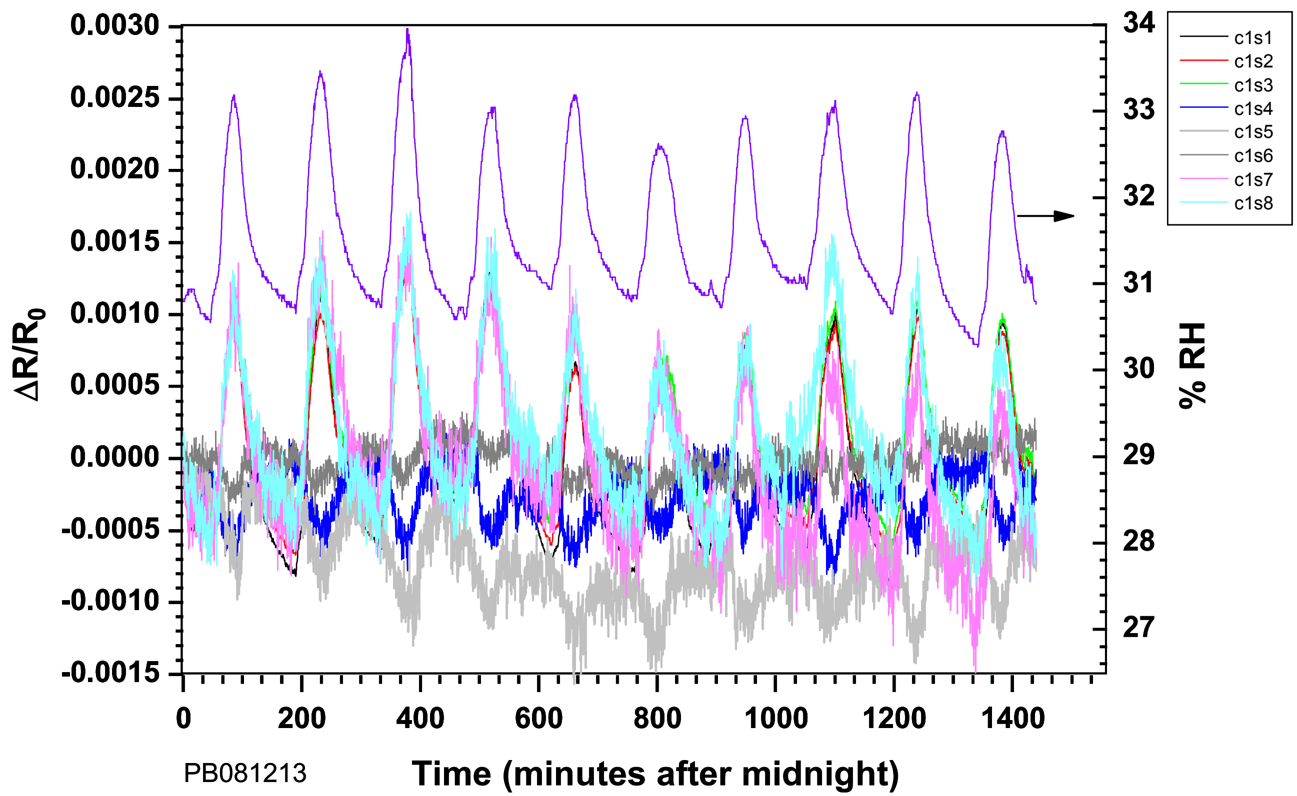
The ENose deployed on EXPRESS Rack 2 on ISS. ENose has been working normally since activation on Dec 9, 2008. The picture on the left shows the green power light is on. The screen is dimmed, and so does not show the time, humidity and pressure in this picture. The screen can be “woken up” by pressing one of the front panel keys.

JPL ENose on the ISS:
Data for 13 Dec. 2008, humidity cycles

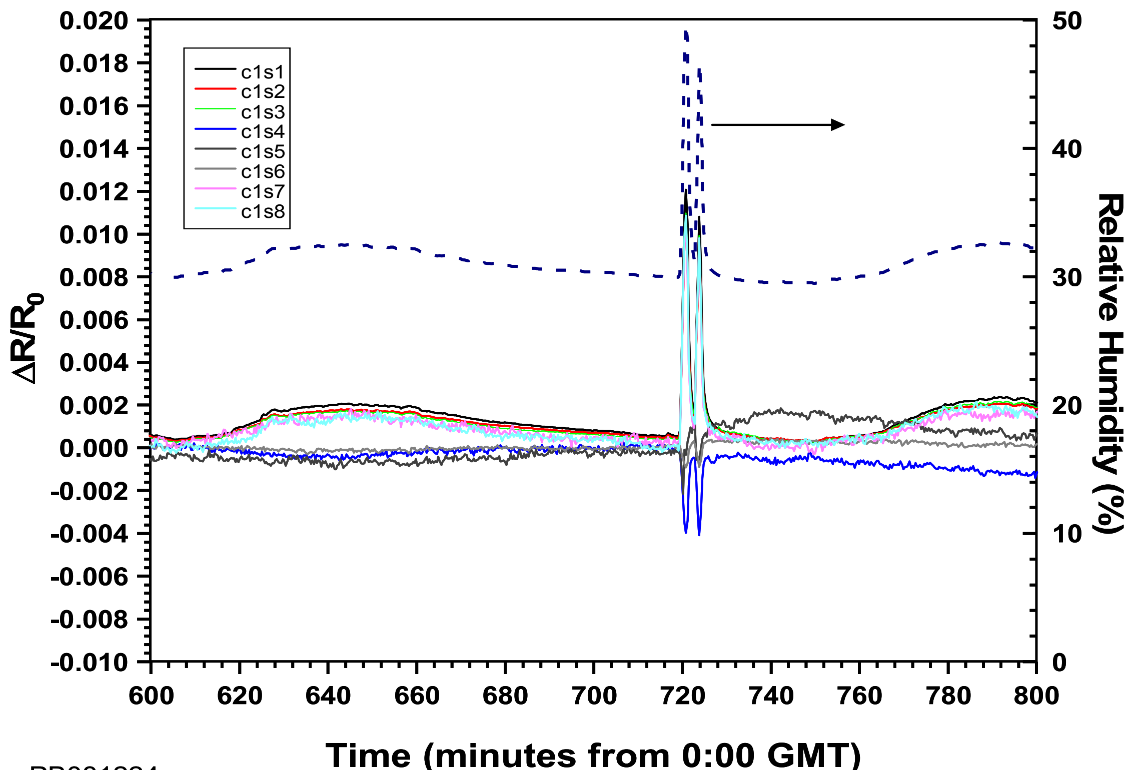


ENose sensor data for December 13, 2008; eight sensors plotted with change in resistance against the initial point in the trace. The violet trace on the top is relative humidity as measured by a humidity sensor in the sensing chamber of ENose (right axis); the 144-minute periodic change in humidity (~3% RH) is attributed to the Carbon Dioxide Removal Assembly, which has a 144 minute cycle.

JPL ENose on the ISS:

Data Analysis of Confirmational Event

A confirmational event is done once every two weeks (disinfectant wipe, dimethyl benzyl ammonium chloride dissolved in water.) This event is evident in the data and is interpreted as an unknown event by the ENose analysis. The signature of the wipe was not included in the data library so we could check the ability of the ENose to report unknowns.



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ENOSE Technology Demonstration: *ISS Data Analysis Summary*

Species	No. of Events	Min Con (ppm)	Max Con (ppm)	Possible Source
Ethanol	1	450	800	cleaning solvent
Methanol	22	3	40	cleaning solvent
Formaldehyde	54	0.18	0.22	exercise equipment
Freon 218	18	6	91	coolant (Russian module)
Unknown	22	-	-	-
Confirm. Event	13	-	-	-

- No event lasted longer than 2 hours. Most were 20 – 60 minutes, consistent with air refresh rate in US Lab
- No identified event exceeded Spacecraft Maximum Allowable Concentration (SMAC) for the time it lasted
- Formaldehyde events could not be correlated with crew exercise; lack of as-performed schedule data
- Unknown was identified by using sensor-analyte interaction models

JPL ENose on the ISS:
Summary of events

A few other events have been detected:

Ethanol

Dec 28, 2008, 10:00 to 10:30 GMT
800 ppm

Jan 01, 2009, 09:44 to 10:26 GMT
440 ppm

Methanol

Jan 03, 2009 11:17 to 12:01 GMT
3-10 ppm

Jan 17, 2009 18:47 to 19:05 GMT
3 ppm

Formaldehyde

Jan 17, 2009, 14:34 to 15:24 GMT
0.15 to 0.20 ppm

Unknown (not confirmational event)

Dec 28, 2008, 16:54 to 17:12 GMT

Jan 09, 2009, 13:28 to 14:34 GMT

Jan 13, 2009, 11:02 to 11:30 GMT

Jan 13, 2009, 20:37 to 21:52 GMT

Jan 15, 2009, 23:01 to 23:24 GMT

Jan 27, 2009, 12:23 to 12:53 GMT

Jan 27, 2009, 21:27 to 23:18 GMT

JPL ENose on the ISS: *Event - Activity Correlation*

- **ENose events detected on ISS could not be confirmed**
  - lack of as-performed activity data
  - lack of other chemical analysis data
- **ENose operation in Environmental Control & Life Support Systems Module Simulator might help in developing an understanding of how ENose responds to regular activities**
  - activities are recorded by test volunteers
  - on-line, continuous chemical analysis for some species
- **Arranged to operate ENose in Regenerative Environmental Control & Life Support Systems Module Simulator (REMS) at MSFC in spring-summer 2009**

ACKNOWLEDGMENTS

The JPL ENose team thanks Babak Kateb and Mike Chen of City of Hope Cancer Center for initiating studies of use of the ENose in tumor detection.

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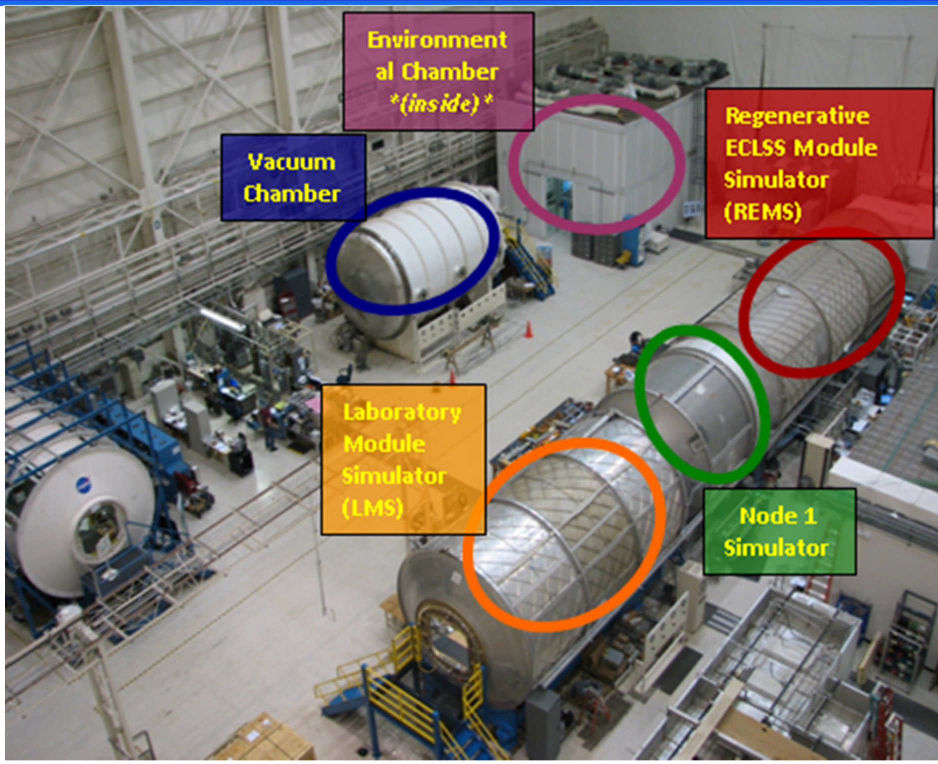
The Regenerative ECLSS Module Simulator



Human-in-the-Loop testing is done in the REMS to test and characterize the Environmental Control and Life Support System (ECLSS) used on ISS. ENose was installed for 3 months while simulated crew activities such as exercise were undertaken in the chamber.

Regenerative Environmental Control & Life Support Systems Module Simulator

Operating Parameters

- **Ambient Pressure**
- **Temperature**
  20 - 22.5 °C (68 °F - 72 °F)
- **Volume**
  ~ 160 m^3 (5660 ft^3)
- **Condensate generation**
  ~27 kg/day (60 lb. per day)

Installed space station subsystems

- **Common Cabin Air Assembly**
  with "flight like" heat exchanger
- **Exercise Equipment**
  four treadmills
  one stationary bicycle
  two elliptical trainers

The JPL ENose Team

Amy Ryan, Principal Investigator
Margie Homer, Co-Investigator
Abhijit Shevade, molecular modeling
Adam Kisor, mechanical, testing
April Jewell, sensor fab and testing
Hanying Zhou, data analysis
Christine Pelletier, data analysis
Ken Manatt, electronics, mechanical
Shiao-Ping (Elizabeth) Yen, polymers

Alex Ksendzov, Optical resonator
Chuck Taylor, visiting prof, Pomona College

Julia Torres (Glendale Comm. College)
Jessica Soler (GCC)
George Hernandez (GCC)
Nathan Sutter (Cal State Northridge)

REMS EXPERIMENT

- **Volatile organic contaminants introduced continuously on working days:**

  6.57 mL/min, 70% ethanol, remainder

acetaldehyde	methanol
acetone	*i*-propanol
benzyl alcohol	propylene glycol
dichloromethane	*m*-xylene

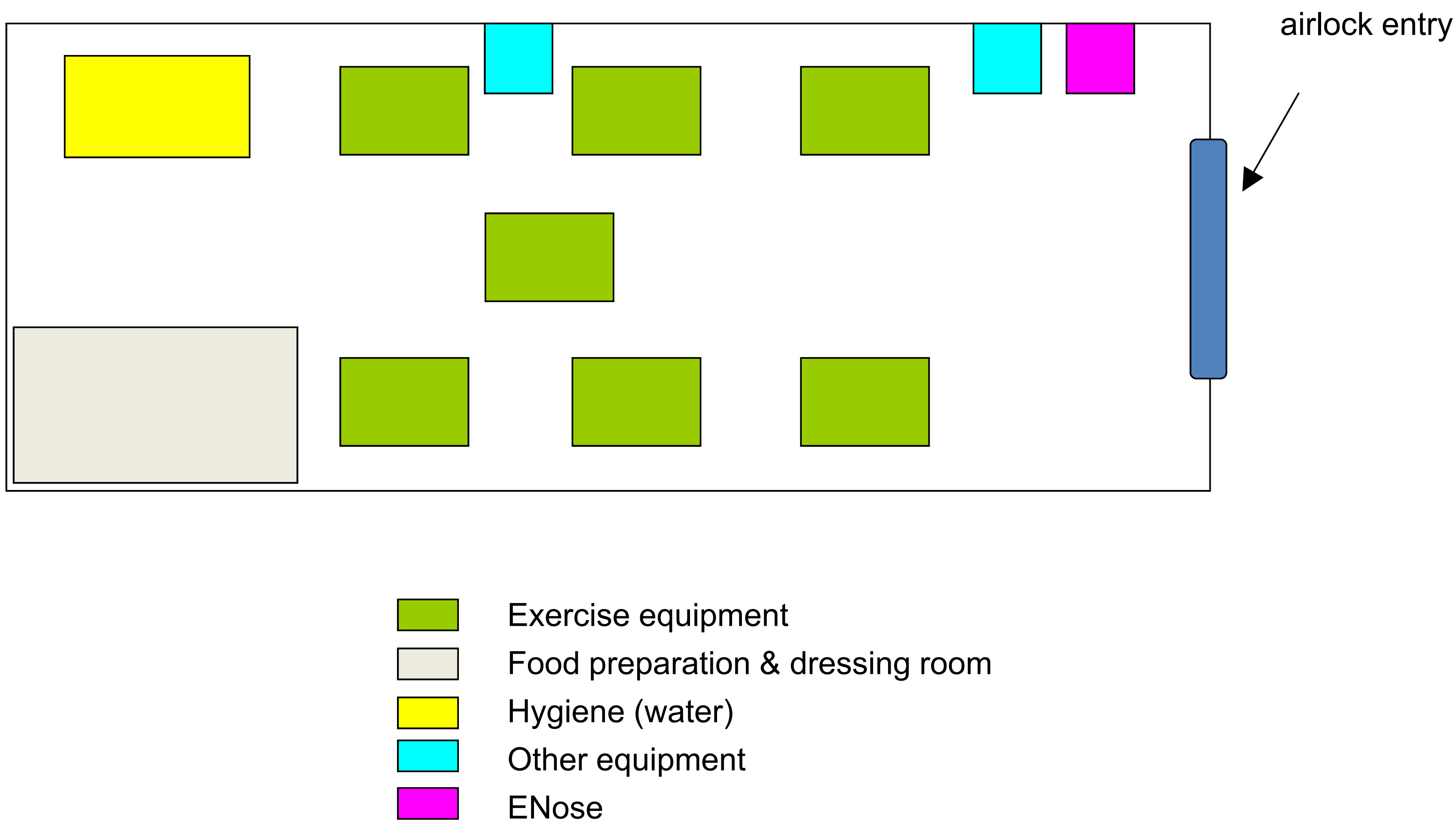
- **Air Monitoring: Continuous analysis by FTIR and GC**

carbon monoxide (CO)	ethane
carbon dioxide (CO_2)	ethanol
	methane

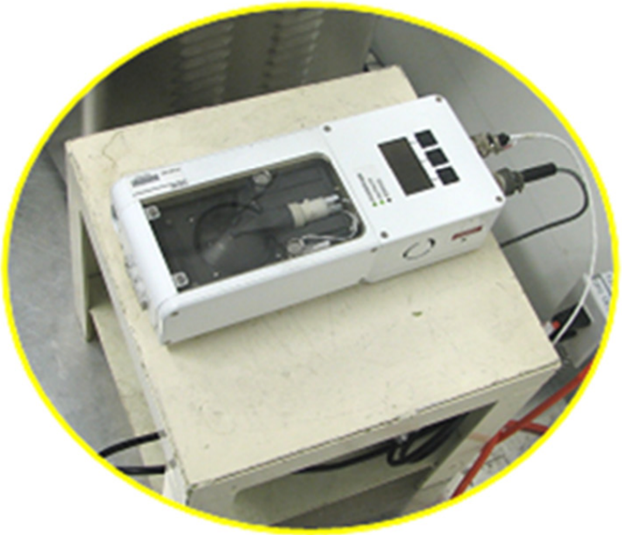
ACTIVITIES IN REMS

- **Several test volunteers enter REMS daily to**
  - exercise on equipment
  - hygiene activities
    - oral hygiene
    - washing (hands, full body)
    - shaving
  - cooking food (frozen dinners)
  - eating dinner
  - cleaning with hygiene wipes
- **Activities, weight change after exercise and use of water are logged for condensate information**
- **Exercise clothing is hung up to dry in REMS after exercise**

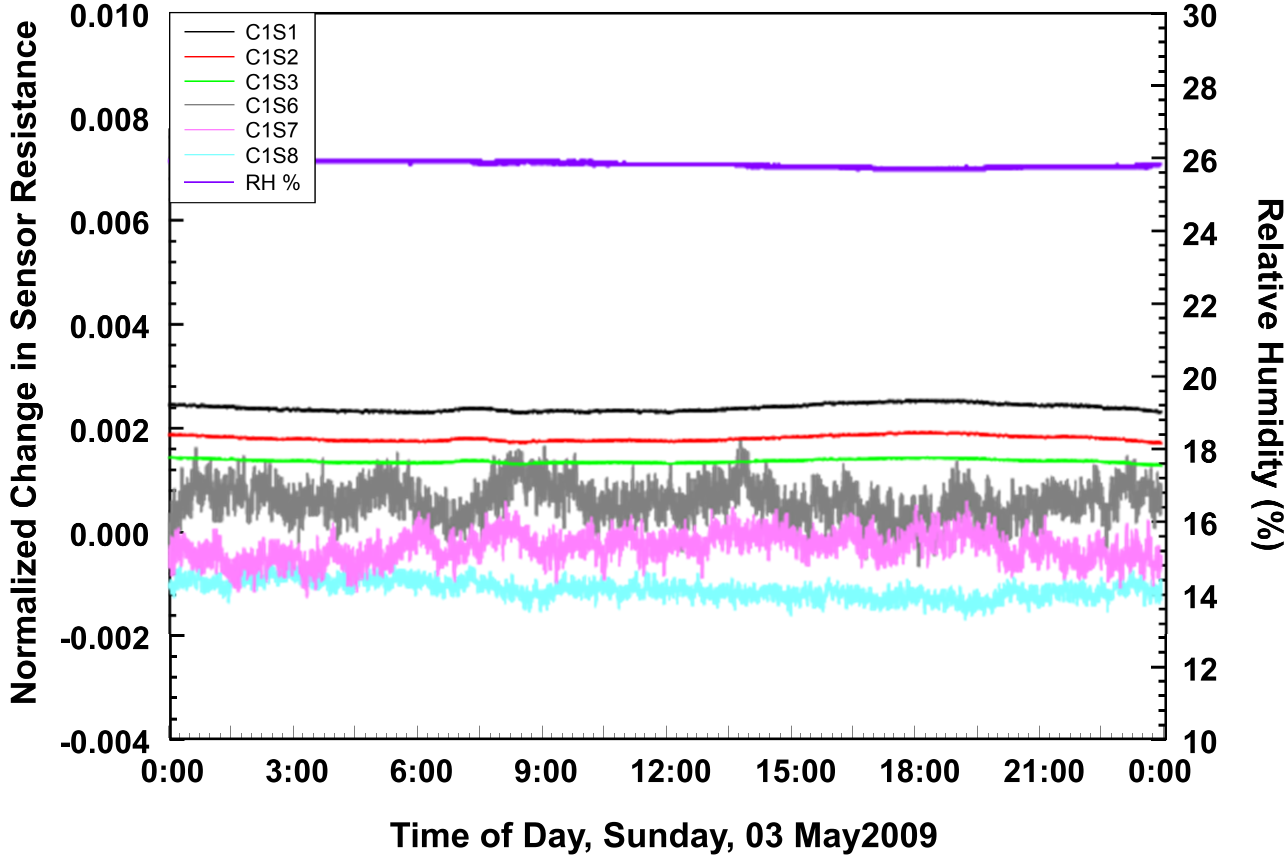
REMS LAYOUT


airlock entry
Exercise equipment
Food preparation & dressing room
Hygiene (water)
Other equipment
ENose

ENOSE EXPERIMENT IN REMS



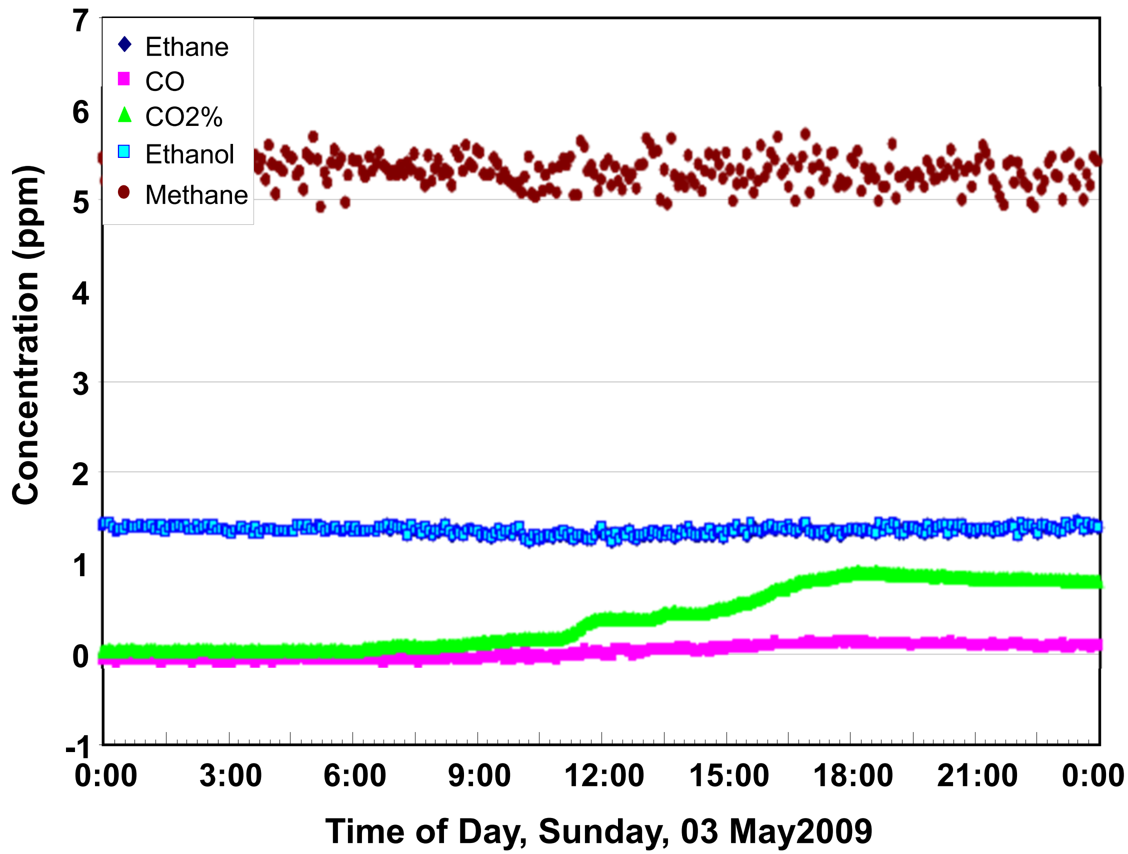
ENOSE EXPERIMENT IN REMS



No activity on weekends and holidays

ENose sensor responses on Sunday, May 3, show no significant changes in air composition. Top trace is relative humidity. ENose Sensor traces have been separated.

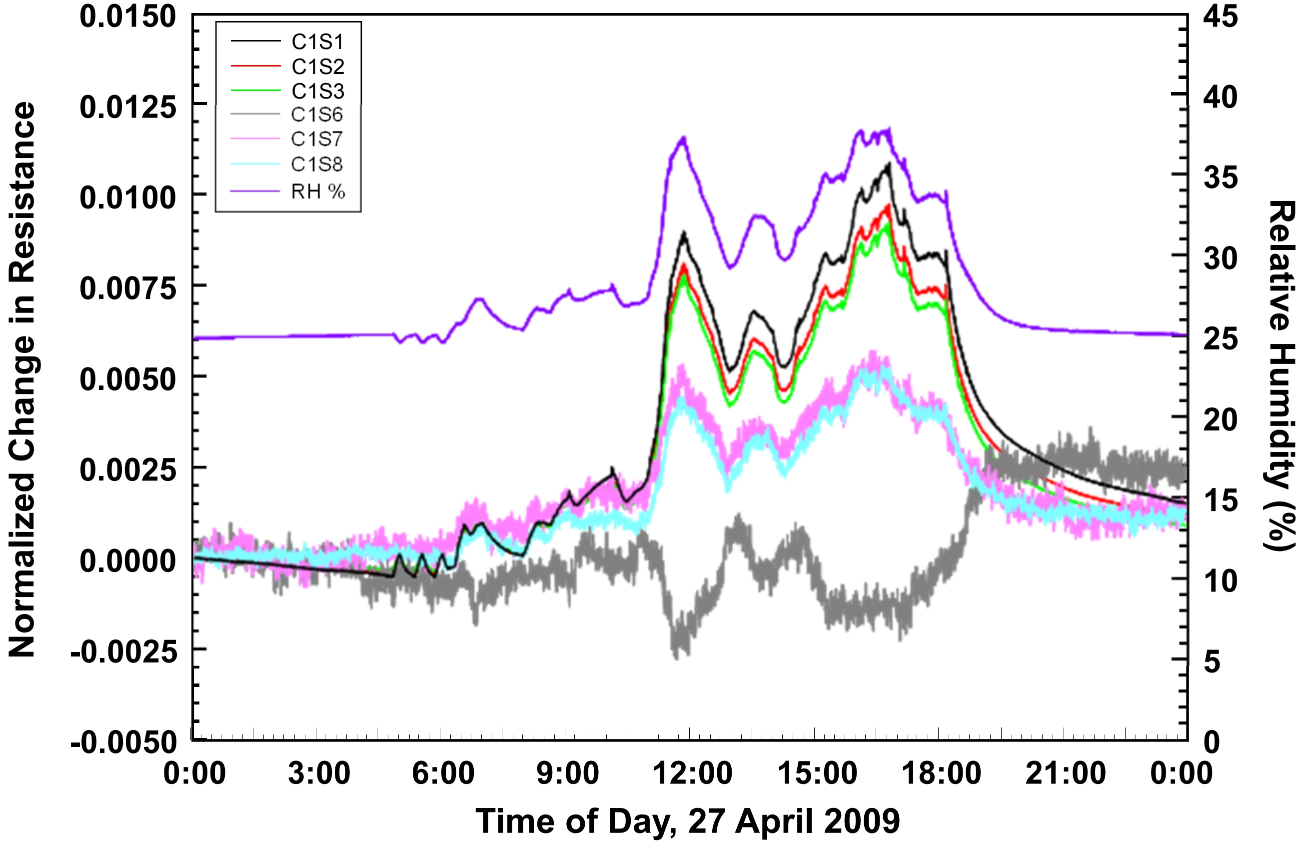
FTIR DATA FROM REMS



No activity on weekends and holidays

FTIR data response on Sunday, May 3, shows no significant changes in air composition.

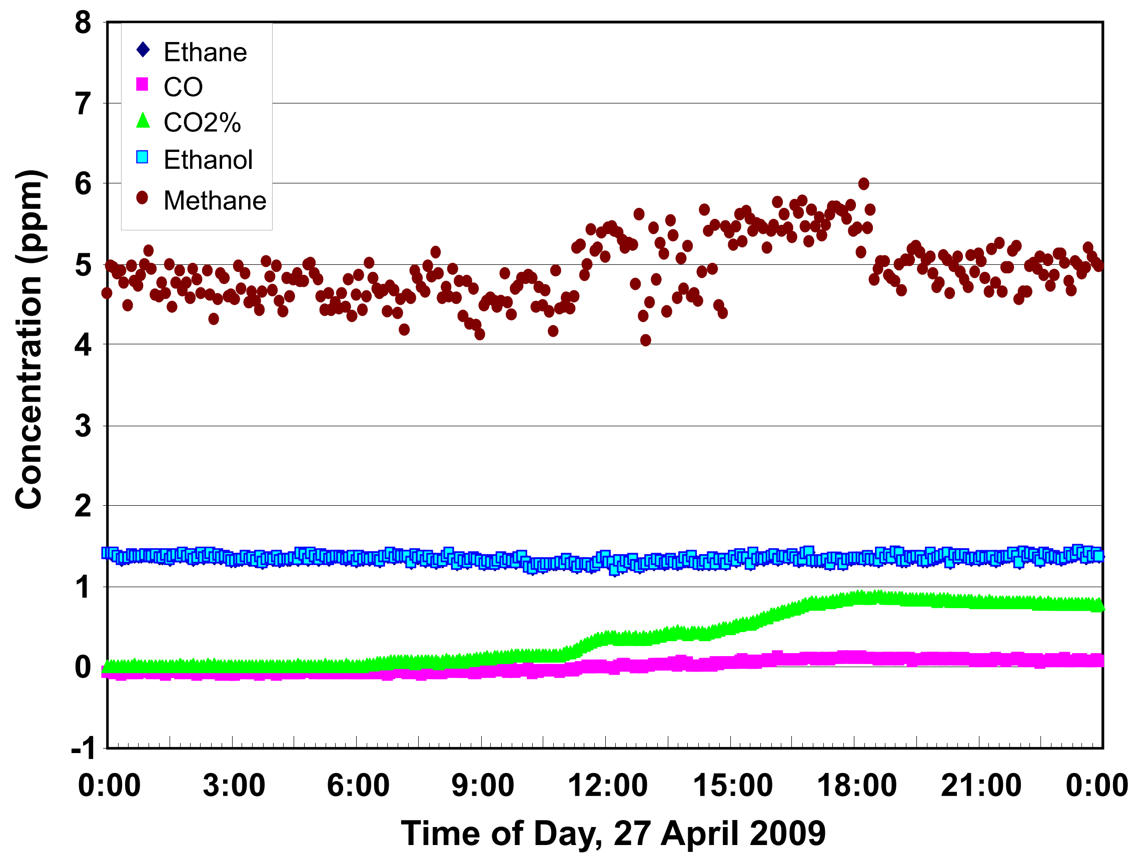
ENOSE DATA FROM REMS



Activity in REMS throughout the working day

ENose sensor responses for Monday, April 27, show changes in relative humidity and air composition starting in the morning and through the workday.

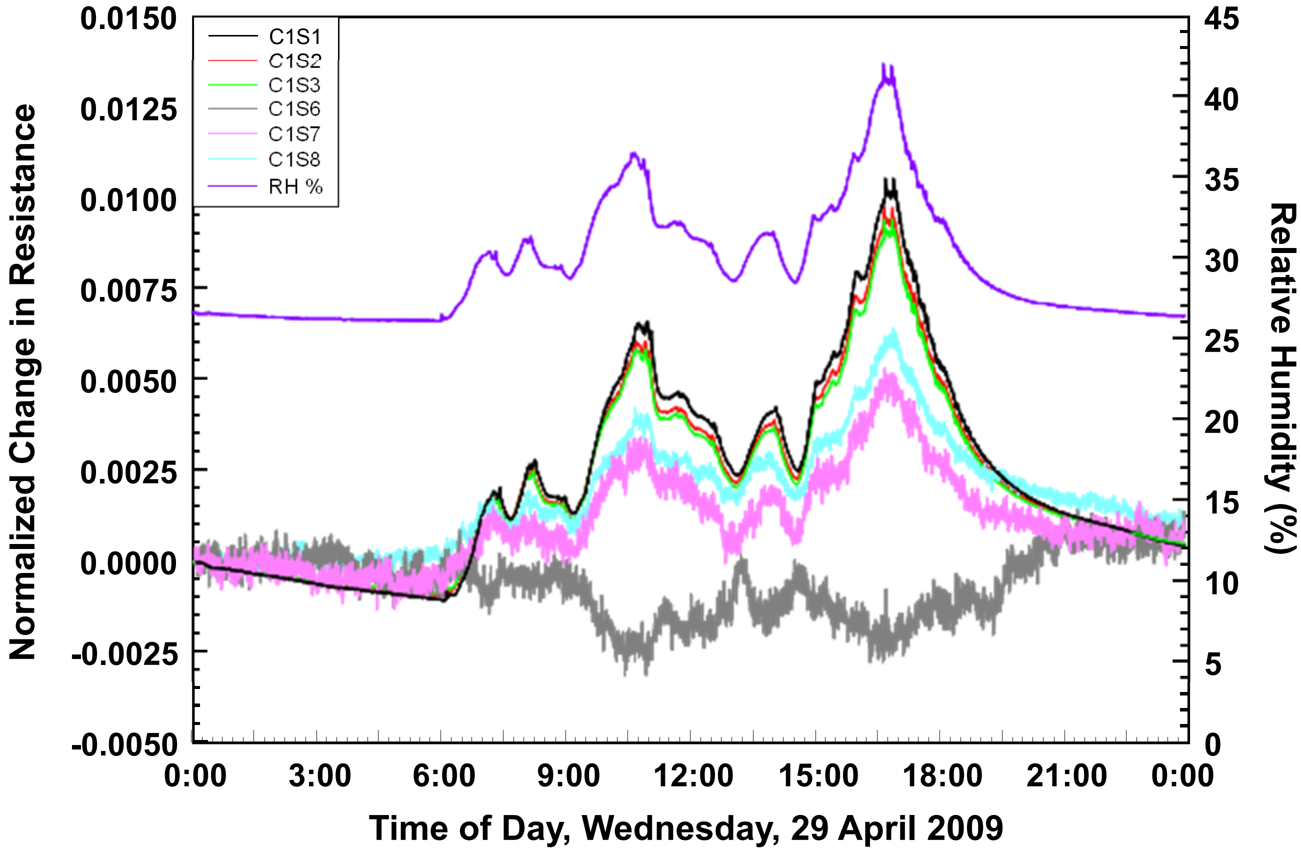
FTIR DATA FROM REMS



Activity in REMS throughout the working day

FTIR data for Monday, April 27, show changes in methane and ethanol starting in the morning and staying up through the workday.

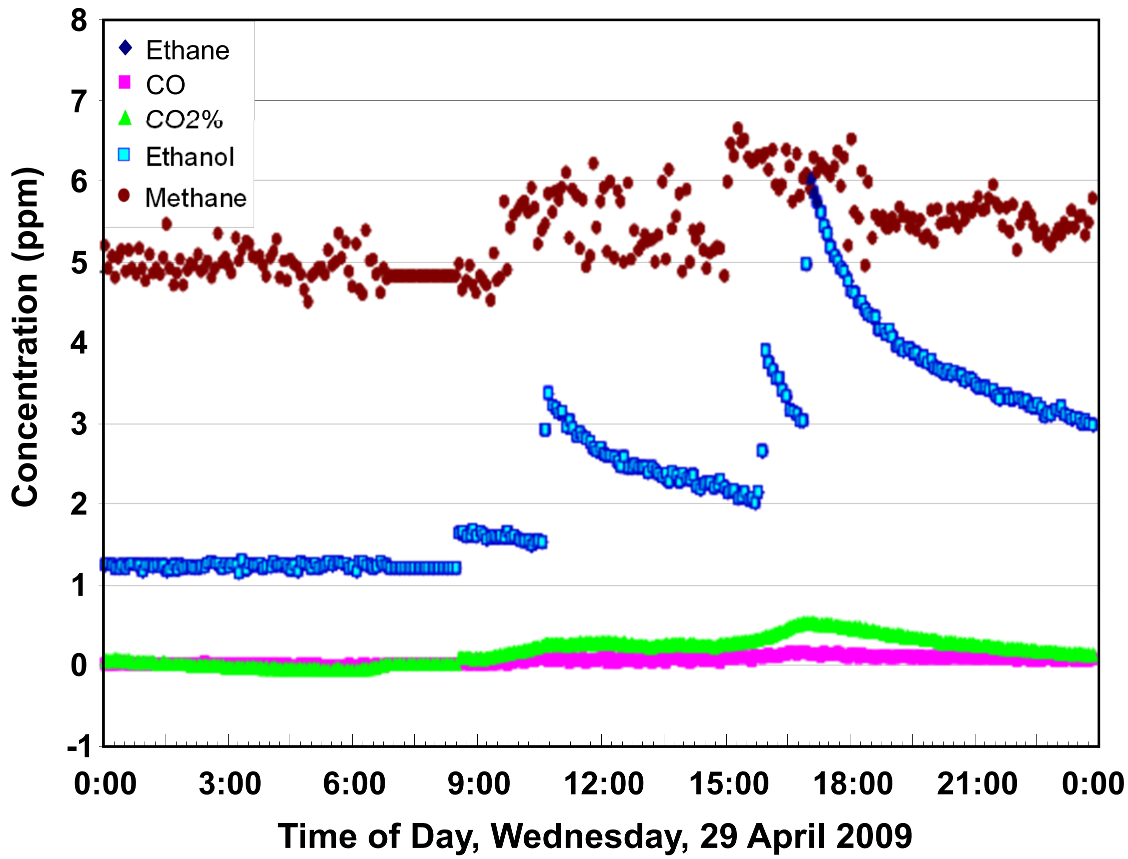
ENOSE DATA FROM REMS



Activity in REMS throughout the working day

ENose sensor responses for Wednesday, April 29, show changes in relative humidity and air composition starting in the morning and through the workday.

FTIR DATA FROM REMS



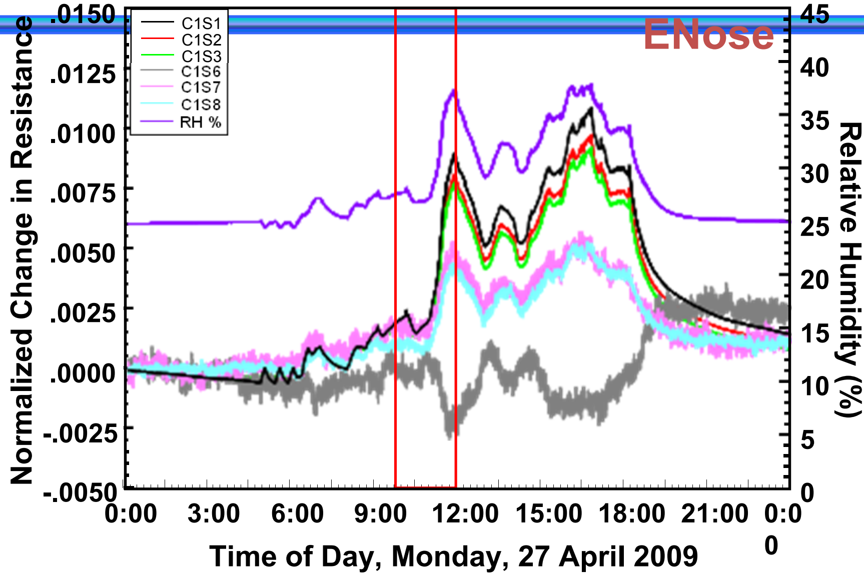
Activity in REMS throughout the working day

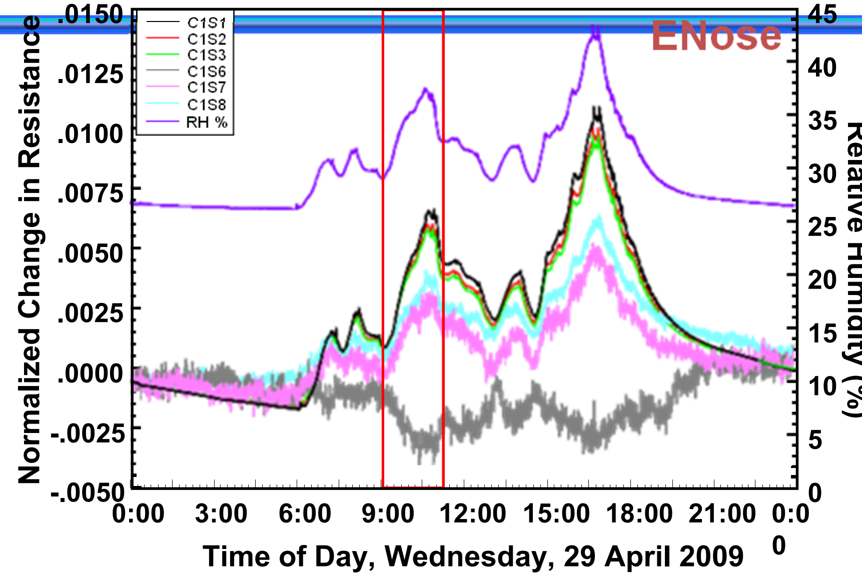
FTIR data for Wednesday, April 29, show changes in methane and ethanol starting in the morning and staying up through the workday.

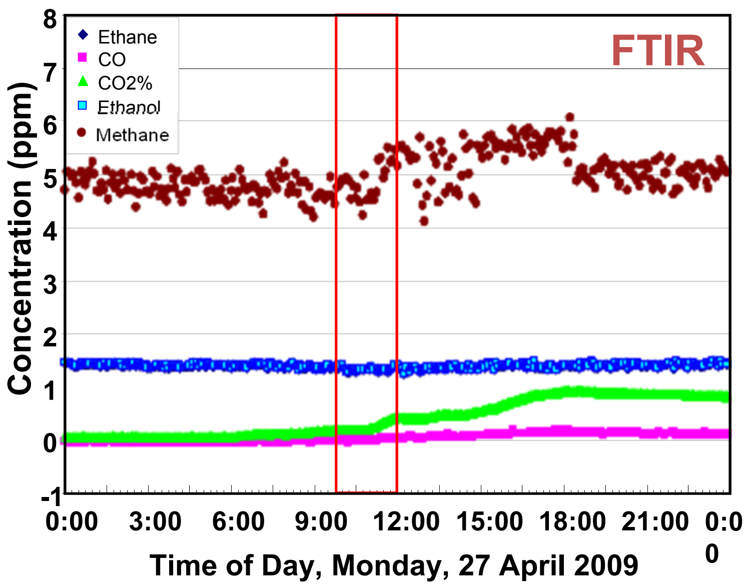
ENOSE DATA FROM REMS

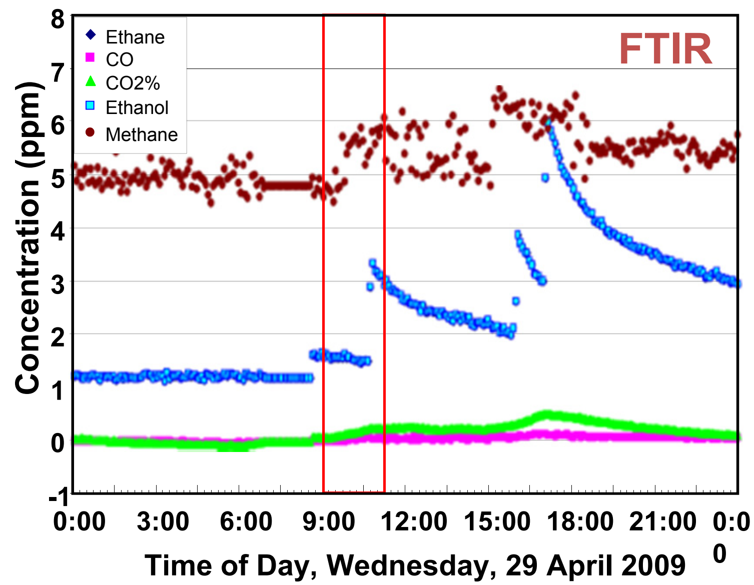
	Event	ppm (±10%)	Start Time	End Time	Duration
April 27	Ethanol	1526	09:57	11:59	2:02
April 28	unknown	--	13:32	13:50	0:18
April 29	Ethanol	758	09:12	11:03	1:51
April 30	--				
May 1	Ethanol	882	14:18	16:16	1:58
May 4	unknown	--	18:46	19:49	1:03
May 5	--				
May 6	Ethanol	1036	09:22	11:29	2:07
	unknown	--	19:33	20:05	0:32
May 7	--				
May 8	Ethanol	1537	07:26	09:28	2:02
	SO_2	0.57	18:17	18:45	0:28

ENOSE & FTIR DATA FROM REMS

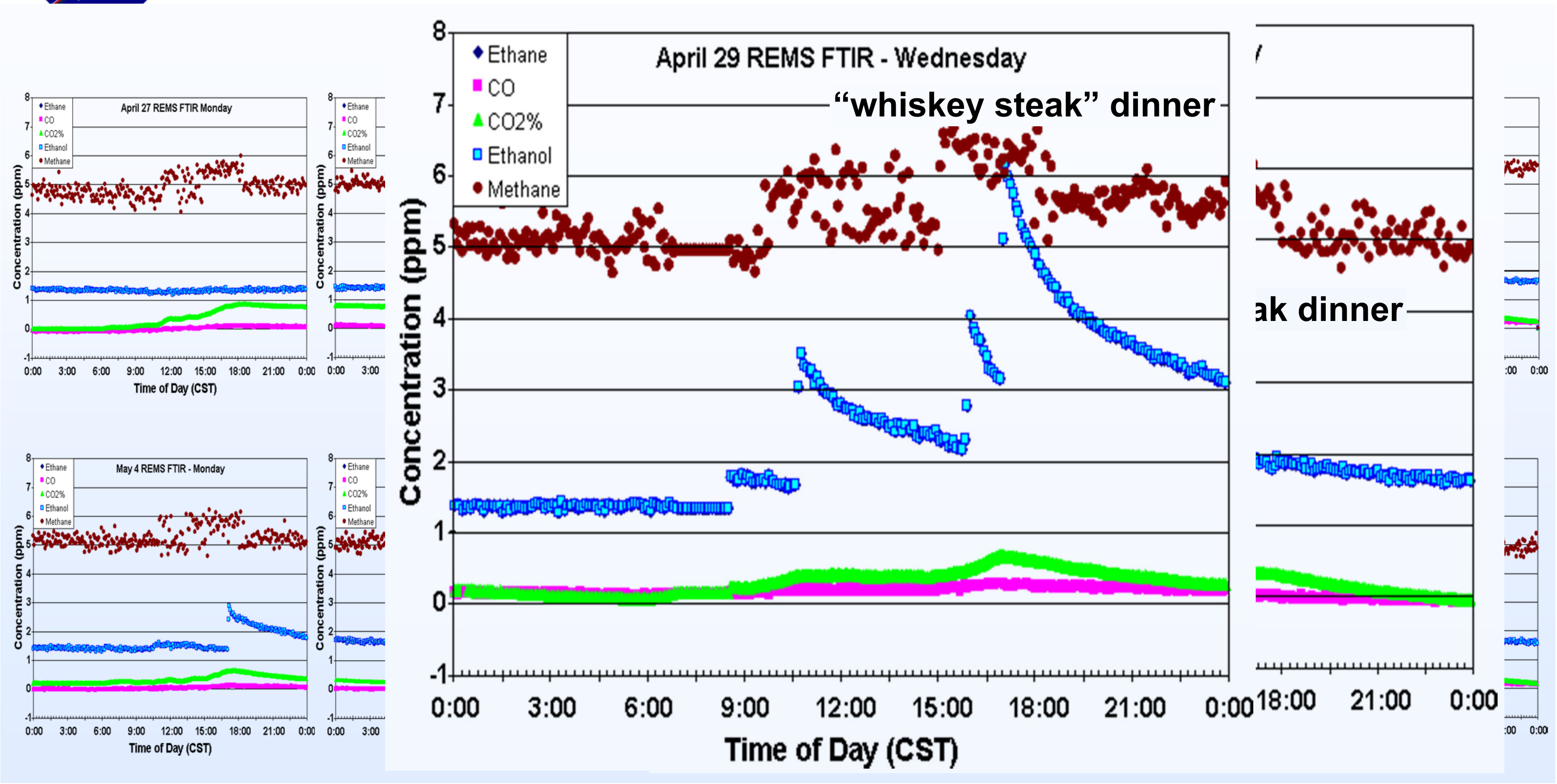




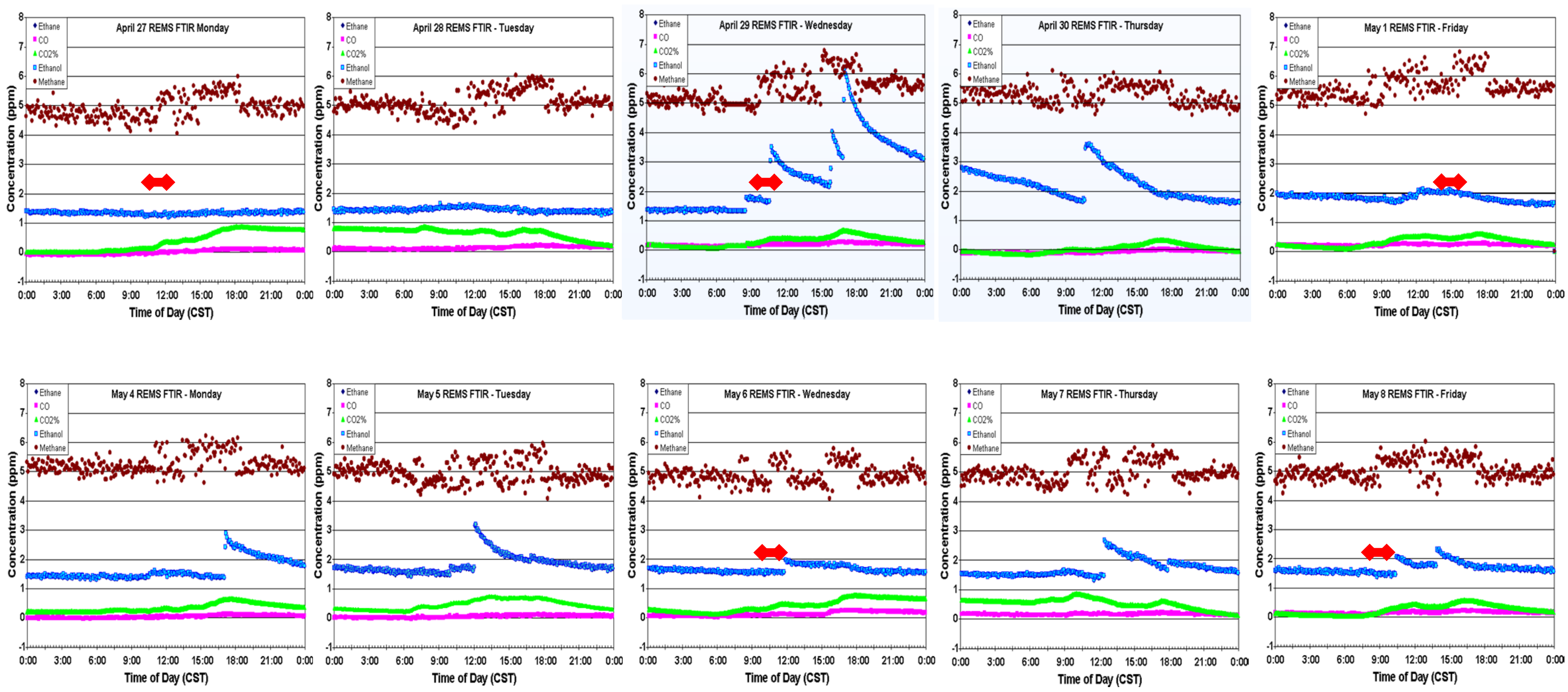




FTIR ANALYSIS IN REMS



FTIR ANALYSIS IN REMS



ENose detects ethanol event

ENOSE EXPERIMENT IN REMS

ENose detected several events analyzed as ethanol release

ENose detected ethanol				Test Volunteer #9 exercised		
Monday	Apr 27	1526 ppm	10 am	Monday	Apr 27	9 am
Tuesday	Apr 28	--		Tuesday	Apr 28	--
Wednesday	Apr 29	758 ppm	9 am	Wednesday	Apr 29	9 am
Thursday	Apr 30	--		Thursday	Apr 30	9 am
Friday	May 1	882 ppm	2 pm	Friday	May 1	12 pm
Saturday	May 2	--		Saturday	May 2	--
Sunday	May 3	--		Sunday	May 3	--
Monday	May 4	--		Monday	May 4	NA
Tuesday	May 5	--		Tuesday	May 5	10 am
Wednesday	May 6	1036 ppm	9 am	Wednesday	May 6	9 am
Thursday	May 7	--		Thursday	May 7	6 pm
Friday	May 8	1537 ppm	8 am	Friday	May 8	9 am
Saturday	May 9	--		Saturday	May 9	--
Sunday	May 10	--		Sunday	May 10	--

ENOSE EXPERIMENT IN REMS

- ENose detected 5 ethanol events April 27 – May 8
- All ethanol events correlate in time with periods that Test Volunteer #9 was in the REMS
- No correlation with activities other than exercise
- Ethanol events were not detected every time that Vol.9 exercised
- Ethanol events detected by ENose do not correlate with ethanol peaks in FTIR

INTERVIEW WITH TEST VOLUNTEER #9

- Vol.9 used two exercise machines on alternate days
- One machine was next to ENose; one at the other end of REMS
- Vol.9 was using two topical medications with ethanol base

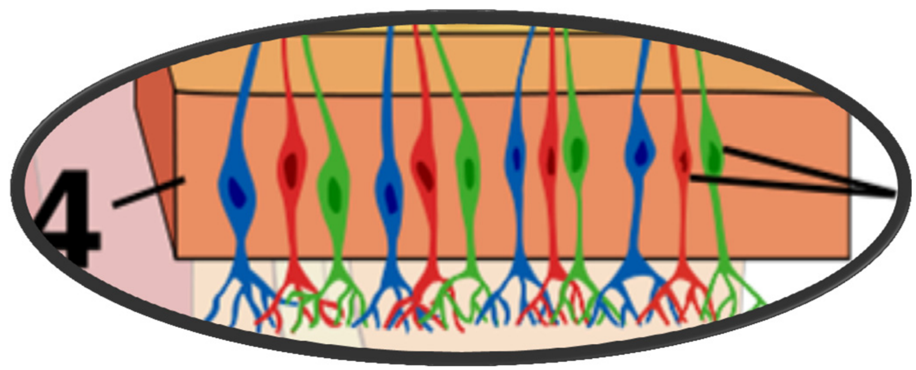
NASA'S INTEREST IN MEDICAL APPLICATIONS

LONG-DURATION SPACE FLIGHT

- Crews of 2-5 persons for 3 months to 2 years
  - ISS missions 3 months
  - Moon missions 6-12 months
  - Mars missions ~ 2 years
- ISS and Moon missions have 1-4 day return time in medical emergency; Mars missions have 7-12 month return time.
- Dependence on local diagnosis and care. One crew member may be MD.
- NASA requires development of small, low power diagnostic and treatment aids for use in local care and in telemedicine

ELECTRONIC NOSE

In the last few years, there have been several *in vitro* and *in vivo* studies of diagnosis using an electronic nose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